MYCORRHIZAL RESPONSE OF POTATO PLANTS TO HOMOKARYOTIC VERSUS DIKARYOTIC ARBUSCULAR MYCORRHIZAL FUNGI.

VICTORIA CATHERINE TERRY

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Department of Biology
Faculty of Science
University of Ottawa

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts that colonize the roots of the majority of vascular land plants. These fungi have a unique nuclear organization, in which thousands of nuclei co-exist among an unsegmented fungal body. In individual strains these nuclei can all be genetically similar (homokaryotic) or be derived from two distinct parents (dikaryotic). In other fungal groups the presence of two distinct nuclei in one cell (fungal dikaryons) can change their fitness, function, and symbiotic relationship; begging the question, what impact does the presence of two parental genotypes have on the arbuscular mycorrhizal symbiosis? I am investigating this by measuring the mycorrhizal response (MR) of potato cultivars with different degrees of domestication using representative AMF homokaryons (4) and AMF dikaryons (4). I found that the genetic organization (dikaryotic vs homokaryotic) and domestication status of the host (modern vs old) are both significant factors in the mycorrhizal response of host plants. Specifically, biomass is significantly greater when inoculated with homokaryotic AMF compared to dikaryotic AMF. Dikaryotic strains have low arbuscule colonization in modern cultivars and higher in old, although there are not significant differences in other fungal responses between homokaryotic and dikaryotic AMF. Furthermore, nutrient uptake (N and P) is greater in old cultivars than modern cultivars, although the root:shoot ratio is lower in old cultivars. Analyses of single spores using digital droplet PCR (ddPCR) confirm that nucleotype ratio of dikaryotic spores shifts depending on the host identity. This research provides novel insights into the role of AMF genetic organization in the mycorrhizal symbiosis in greenhouse conditions. In particular, this work shows that the presence of two distinct nucleotypes results in the fungi being more readily adaptable to the host leading to a more stable MR and a potentially selfish strategy, when in symbiosis with potato cultivars.

RESUME

Les champignons mycorhiziens arbusculaires (AMF) sont des symbiotes végétaux obligatoires qui colonisent les racines de la majorité des plantes terrestres vasculaires. Ces champignons ont une organisation nucléaire unique, dans laquelle des milliers de noyaux coexistent au sein d'un corps fongique non segmenté. Dans les souches individuelles, ces noyaux peuvent tous être génétiquement similaires (homocaryotes) ou provenir de deux parents distincts (dicaryotes). Dans d'autres groupes fongiques, la présence de deux noyaux distincts dans une cellule (dicaryons fongiques) peut modifier leur forme physique, leur fonction et leur relation symbiotique; pour poser la question, quel impact la présence de deux génotypes parentaux a-t-elle sur la symbiose mycorhizienne arbusculaire? J'étudie cela en mesurant la réponse mycorhizienne (MR) de cultivars de pomme de terre avec différents degrés de domestication en utilisant des homocaryons AMF représentatifs (4) et des dikaryons AMF (4). J'ai découvert que l'organisation génétique (dicaryote vs homocaryote) et le statut de domestication de l'hôte (moderne vs ancien) sont tous deux des facteurs importants dans la réponse mycorhizienne des plantes hôtes. Plus précisément, la biomasse est significativement plus élevée lorsqu'elle est inoculée avec l'AMF homocaryote par rapport à l'AMF dicaryote. Les souches dicaryotes ont une faible colonisation par les arbuscules dans les cultivars modernes et plus élevée dans les anciens, bien qu'il n'y ait pas de différences significatives dans les autres réponses fongiques entre les AMF homocaryotes et dicaryotes. De plus, l'absorption des éléments nutritifs (N et P) est plus élevée chez les anciens cultivars que chez les cultivars modernes, bien que le rapport racine:pousse soit plus faible chez les anciens cultivars. Les analyses de spores individuelles à l'aide de la PCR de gouttelettes numériques (ddPCR) confirment que le rapport de nucléotype des spores dicaryotes change en fonction de l'identité de l'hôte. Cette recherche fournit de nouvelles informations sur le rôle de l'organisation génétique du AMF dans la symbiose

mycorhizienne en conditions de serre. En particulier, ce travail montre que la présence de deux nucléotypes distincts rend les champignons plus facilement adaptables à l'hôte, ce qui conduit à une MR plus stable et à une stratégie potentiellement égoïste, lorsqu'ils sont en symbiose avec des cultivars de pomme de terre.

TABLE OF CONTENTS

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LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AMF – Arbuscular mycorrhizal fungi

AM – Arbuscular mycorrhizal

DIK – Dikaryon

ddPCR – droplet digital polymerase chain reaction

HOM – Homokaryon

MAT – Mating type

MR – Mycorrhizal response

N – Nitrogen

P – Phosphorus

ROC – Root organ culture

Tuber No. – Tuber number

CHAPTER ONE

Introduction

1.1 Overview of Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi (subphylum Glomeromycotina) are root symbionts thought to have facilitated plants' colonization of land and their diversification across ecosystems over 400 million years ago (Selosse and Tacon, 1998; Wang and Qiu, 2006; Bonfante and Selosse, 2010). Approximately 327 species are described to date (Błaszkowski et al., 2022; "International Culture Collection of Glomeromycota [CIGC]," 2022; Stürmer and Kemmelmeier, 2021), each with the ability to form symbioses with a diverse array of plant hosts, often simultaneously. They colonize approximately 80% of all land plants (Smith and Read, 2008) including the majority of economically important crops and are globally widespread, existing on every continent (Savary et al., 2018).

Having coevolved with plants, AMF are obligate symbionts, meaning they require a plant host and the carbon (sugars, lipids) it provides them (Smith and Read, 2008). In exchange, AMF increase the success of the plants by reaching and improving acquisition of soil nutrients (i.e., phosphates and nitrates) and water inaccessible by roots to the plant in exchange for photosynthetically fixed carbon sources (Bonfante and Genre, 2010; Smith and Read, 2008). In addition to this, AMF increase plant resistance to biotic and abiotic stressors such as pathogens, herbivores, and parasites (Jung et al., 2012a), toxins (Mitra et al., 2022), and drought (Liu et al., 2018). Because of these benefits, AMF have increasingly been used and studied in the agriculture industry with the aim of increasing plant productivity and reducing the demand for chemical fertilizer (Berruti et al., 2016; Hamel, 1996).

1.2 Biology of AMF

1.2.1 Unique physiology

The mycelium of AMF is made up of a network of branching tube-like filaments called hyphae that are formed by cell walls that are high in chitin (Kokkoris et al., 2020; Smith and Read, 2008). Extraradical hyphae spread an extensive mycelial network throughout the soil microbiome (Brachmann and Parniske, 2006). The extraradical hyphae form spores which store carbon in the form of lipids and have a protective multilayer wall containing chitin, which allows the spores to be viable for several years and used as propagules (Müller et al., 2017; Smith and Read, 2008).

Before coming into physical contact, the host plant and the AMF communicate using signaling molecules (Lanfranco et al., 2018). For example, the plant releases strigolactones which induce germination and hyphal branching of the AMF, increasing the probability of connecting with the host (López-Ráez et al., 2011). Signaling strigolactones are increased when the plant is low in phosphorus, seemingly acting as a 'cry for help' by the plant to the AMF (Lanfranco et al., 2018). Similarly, there are mycorrhizal signalling molecules released by the AMF, in particular lipooligosaccharides and chitin oligomers which induce branching in the host roots and increase the chance of contact with the host (Genre et al., 2013; Maillet et al., 2011).

When in contact with plant roots, the hyphae form adhesion structures (hyphopodia) on the root epidermis (Smith and Read, 2008). The hyphae then penetrate the roots (intraradical hyphae; fig. 1.1) extending both intercellularly and intracellularly (Smith and Read, 2008). Here they can form vesicles which, similarly to spores, store lipids and may act as propagules (Müller et al., 2017). In the cortex of the root, the hyphae enter single cells, where they form highly branched, tree-like structures called arbuscules (Gutjahr and Parniske, 2013; Lanfranco et al.,

2018; Smith and Read, 2008). Plant-derived peri-arbuscular membrane surrounds the arbuscule and nutrient is exchanged between the AMF and host across these two structures (Brachmann and Parniske, 2006).

1.2.2 Unique nuclear biology

AMF present a highly unique cellular biology. They are constantly multinucleate and coenocytic (non-septate hyphae), meaning that hundreds to thousands of nuclei flow throughout the cytoplasm of a large unsegmented fungal body (the mycelium) (Kokkoris et al., 2020). Therefore, the coenocytic hyphae are one large cell. In the model species, *Rhizophagus irregularis*, strains are found to be either dikaryon-like (or AMF dikaryons; fig. 1.2); containing hundreds of nuclei derived from two parental strains (two parental nucleotypes) or homokaryotic; containing hundreds of nuclei of one genotype (E. C. H. Chen et al., 2018; Ropars et al., 2016). Within AMF dikaryons, the two nucleotypes contain divergent genomic regions that are similar to the mating type (MAT) loci, which determine sexual compatibility in closely related fungal phyla, Basidiomycota and Ascomycota (Ropars et al., 2016). In these fungal relatives the dikaryotic stage arises as a step in the sexual cycle, followed by karyogamy and meiosis (Clark and Anderson, 2004). However, some fungal species can maintain the dikaryotic stage indefinitely (i.e., never recombine), and this condition may be followed by AMF dikaryons (Debuchy et al., 2014).

1.3 Dikaryosis

1.3.1 Dikaryosis in Ascomycota and Basidiomycota

Without sexual recombination, the dikaryotic state in AMF bodes unknown adaptive advantages and begs the question, why has it arisen? Because the research of AMF dikaryons is in its infancy, we must look to related fungal groups and the way their dikaryotic stage affects their interaction with hosts, to gain insight into the mechanisms that may give the dikaryotic state a 'function.' The dikaryotic strains of the fungal subkingdom Dikarya (i.e., Basidiomycota and Ascomycota) contain two parental nucleotypes, and this genetic condition can affect their phenotype and may significantly impact the strain's fitness and interaction with host organisms (Clark and Anderson, 2004; Parmeter et al., 1963; Strom and Bushley, 2016). For example;

- Dominance effects in one nucleus can mask deleterious alleles in the other nucleus, as seen in wood decay fungus, *Schizophyllum commune,* which exhibits a higher growth rate and greater wood decay capacity in the dikaryotic phase (Amburgey, 1970; Clark and Anderson, 2004).
- Complementation between the two nuclei can occur as seen in the saprobe, *Neurospora crassa*, allowing them to express proteins unique to each nucleotype resulting in increased fitness (Ingold and Hudson, 1993).
- The relative abundance of the two nucleotypes fluctuates, as seen in *Penicillium* sp., where the nucleotype abundance shifts according to the media type, adapting to best fit the environment (Jinks, 1952; Strom and Bushley, 2016).
- Additive effects have been found to increase expression in dikaryotic ectomycorrhizal fungus, *Hebeloma cylindrosporum*, where dikaryons possess higher enzymatic activity

that leads to enhanced nitrogen uptake in host plants compared to either of the homokaryotic parents (Wagner et al., 1988).

- The combination of two divergent nuclei can lead to novel protein interactions in fungal pathogens like *Microbotryum lychnidis-dioicae* and *Ustilago maydis,* in which dikaryosis triggers pathogenicity, making them capable of penetrating the host by inducing filamentation of hyphae that grow inside the plant tissue (Chang et al., 2019; Gillissen et al., 1992; Wallen and Perlin, 2018).

These functions that are enabled by the dikaryotic stage in ascomycetes and basidiomycetes give us possible justification as to why a "dikaryotic state" also emerges in the absence of formal sexual reproduction in AMF and is maintained over many generations.

1.3.2 Dikaryosis in AMF

When we switch our focus back to AMF, there is evidence that these fungi may be incurring similar mechanisms during the dikaryotic stage. A recent study, performed on an *in-vitro* system using root organ cultures (ROCs), demonstrated that the relative abundance of each nucleotype within four dikaryotic strains of *R. irregularis* (A4, A5, SL1 and G1) can be regulated in response to plant host identity (Kokkoris et al., 2021). However, it remains unclear how this impacts gene expression (i.e., if the more abundant nucleotype has greater expression to match). Not long after, it was shown that *R. irregularis* dikaryons have distinct life history strategies compared to the homokaryons, providing them with an adaptive growth advantage (Serghi et al., 2021). In particular, the dikaryotic strains were able to grow faster, produce more complex hyphal networks, and produce more spores across different ROC hosts compared to homokaryotic strains of the species *R. irregularis*. Similar evidence is indirectly present in older

studies where the now known to be dikaryotic strains A4 (Koch et al., 2004) and C3 (Ehinger et al., 2009), produced more spores and higher hyphal density compared to homokaryotic strains. Additionally, while dikaryotic strains seem to be superior in extraradical growth, observations find the opposite, intraradically, with the dikaryotic strain A4 having the lowest intraradical colonization values compared to 12 homokaryotic strains (Savary et al., 2018) further pinpointing significant life history variation between the two genetic organizations.

The distinct growth patterns of fitness-related functional traits in the AMF dikaryons, alongside their higher adaptability on a wider range of hosts (Serghi et al., 2021) and regulation of the abundance of each of the two nucleotypes (Kokkoris et al., 2021), are likely to affect the plant host response. Initial evidence for this can be seen in a greenhouse study on cassava plants (Peña et al., 2020) where the recovery rate of drought-affected plants inoculated with a dikaryotic strain of *R. irregularis* (C3), was significantly higher than that of a homokaryotic strain of the same species, and the plants inoculated with the dikaryotic strain were less susceptible to drought. There is also possibility for less beneficial plant responses due to the increased maintenance cost of more nuclei alongside the increased extraradical growth of the dikaryotic strains which can translate into a strong carbon sink for the plant and the hoarding of beneficial nutrients (e.g., phosphorus and nitrogen) by the fungi.

The plant host response to a variety of homokaryotic and dikaryotic strains, coupled with assessment of the fungal response of dikaryotic AMF *in planta* has not been investigated, but would provide a more comprehensive understanding of how the genetic organization affects this mutualism.

1.4 Nutrient Exchange

Plants are autotrophic, capable of manufacturing their own food, becoming the base of the food chain. Specifically, plants absorb the sun's energy and convert it into chemical energy in the form of ATP and NADPH which is then used to assemble carbohydrate molecules, like glucose, from atmospheric carbon dioxide (Eberhard et al., 2008). The carbohydrate molecules are then transported throughout the plant and used for growth (Génard et al., 2008). This mechanism allows the plant to introduce organic compounds into the ecosystem which then sustain life in other organisms that cannot produce it for themselves (Smith et al., 1969). To perform photosynthesis, the plant requires nutrients obtained from the soil. The most important and often limiting nutrients are phosphorus and nitrogen (Marschner et al., 1996; Marschner, 2011). Both are key elements in ATP and NADPH and therefore, are essential for photosynthesis and carbon fixation (Eberhard et al., 2008). Additionally, nitrogen and phosphorus are essential nutrients required for the development of plant proteins and nucleic acids (Conley et al., 2009).

Phosphorus is predominantly found in the soil as phosphate ions which have low mobility in the soil (Smith et al., 2011). Thus, the plant only has access to the phosphorus surrounding the roots. Once this is used up by the plant, a depletion zone (fig.1.3) is created around the plant and without assistance, from either regular fertilizer input, or soil microorganisms, the plant would be nutrient starved, and unable to continue to complete photosynthesis (Marschner, 2011). This is where AMF comes in. AMF colonizes the roots of plants and acts as an extension of the root system. The microscopic hyphae can access nutrients in tiny crevices where the root cannot reach alone (de Vries et al., 2021; Hodge and Storer, 2014). Additionally, the hyphae extend beyond the depletion zone in broad hyphal networks and quickly transport nutrients from a much greater volume of the soil to the plant (Li et al., 1991). This increases the surface area used for nutrient absorption by more than 100-fold (Hijri, 2016; Smith and Read, 2008). The AMF

provides an effective pathway (AM pathway) for P to be collected and rapidly delivered to the plant cortical cells (Smith et al., 2011).

The relationship between AMF and N acquisition is somewhat less clear, with its contribution to plant N varying widely (Hodge and Storer, 2014). N in the soil is more mobile and thus, more accessible by the plant than P. However, AMF may be better able to access the less mobile forms of nitrogen (i.e., NH4+) that are out of reach to the plant roots (Hodge and Storer, 2014; Sun et al., 2021).

Although specific quantification of nutrient transport to the host by AMF is not widely agreed upon, AMF can sometimes provide the plant with up to 90% of its required N and P (Walder et al., 2012). In exchange for this, the plant gives the AMF approximately 5-10% of its photosynthetically fixed carbon (Hodge and Storer, 2014), in the form of carbohydrates and lipids (Keymer et al., 2017; Smith and Smith, 2012). This exchange can be regulated by each of the symbionts. Host plants reward greater nutrient supply with greater carbon (Bell et al., 2022; Kiers et al., 2011). Similarly, AMF allocates the most P to the host that gives them the most carbon (Kiers et al., 2011). However, this reciprocity seems to be highly context dependent, and plants in some cases invest large amounts of carbon for little return (Walder et al., 2012). Both biotic and abiotic factors seem to play a role in this exchange including the host and AMF identity, nutrient availability, and atmospheric CO2 (Bell et al., 2022; Field et al., 2012; Johnson et al., 2015).

1.5 Mycorrhizal Response

An important measure that defines the mutualistic quality of AMF is known as the Mycorrhizal Response (MR). MR describes any benefit or detriment that the plant exhibits, for a specific trait (e.g., nutrient uptake, biomass etc.) because of inoculation with AMF (Baon et al., 1993; Sawers

et al., 2008). While the symbiosis is at large mutualistic, negative and neutral MR is often observed (Ceballos et al., 2019; Jin et al., 2013; Klironomos et al., 2000; Ryan et al., 2005; Ryan and Angus, 2003).

MR is highly dependent on the plant and fungal identity (Dai et al., 2014; Ehinger et al., 2009; Ryan and Kirkegaard, 2012), with fungal species exhibiting higher intraspecific than interspecific variation (Kokkoris et al., 2019; Munkvold et al., 2004). This may be due to the different strategies of the plant and the AMF between species and strains (e.g., P uptake and C demand; Jones and Smith, 2004), however, other biotic and abiotic factors also affect MR (Hoeksema et al., 2010). For example, MR is found to be less positive under reduced light and it is hypothesized that AMF provides lower benefit when photosynthesis is restricted (Vatovec et al., 2005). Additionally, MR is less positive when phosphorus is in excess and when the plants have nitrogen fixing bacteria, likely because the plant depends less on the AMF to acquire these nutrients (Hoeksema et al., 2010). MR is more positive when the symbiosis occurs in more realistic biotic conditions and with multiple species (Hoeksema et al., 2010). This may be due to complementary effects between species (Hart and Reader, 2002; Maherali and Klironomos, 2007) or the plant being more likely to encounter a highly favourable species (Vogelsang et al., 2006). As well, a higher MR is observed in diverse soil communities, which may be attributed to the benefit of AMF defense against pathogens (Fitter and Garbaye, 1994; Hoeksema et al., 2010).

Although the high variation in MR among AMF strains suggests a single strain could not be optimal for all plant species, the model species *Rhizophagus irregularis* (specifically the strain DAOM-197198) is the main source of inoculum across studies and commercial products. However, there are discrepancies in its effectiveness (Stockinger et al., 2009). For example, some studies report a high positive MR, as seen in potatoes (increased yield and increased tuber

size; Hijri, 2016) while others report negligible responses as seen in peas (Jin et al., 2013). There are even negative responses to inoculation with the commercialized strain DAOM-197198, with several studies observing growth depression as seen, for example, in barley (Christophersen et al., 2009; Grace et al., 2009). These growth depressions are assumed to be caused by the AMF acting as a carbon sink without providing a sufficient increase in P and N uptake to make up for the drain of carbon (Bell et al., 2022; Smith and Read, 2008). However, further investigation is required to gain a complete understanding of this dynamic.

1.6 Wild versus Domesticated Plants

The domestication of plants allowed humans to have continuous access to food and establish food security for a growing population, placing plant domestication and the ensuing agricultural revolution among the great milestones of human civilization (Pérez-Jaramillo et al., 2015; Purugganan and Fuller, 2009). However, the process of plant domestication entails selection for traits of human interest and often ends in plants that are incapable of surviving in the wild (Abbo et al., 2022). After generations of domestication, cultivated plants often rely on humans for survival whether it be for nutrients, water, or reproduction (Abbo et al., 2022). The human driven selection that drives domestication leads to cultivars that are rich in human favoured characters, such as large fruits and other harvested organs, and as a trade-off, poor in those that are not, such as chemical and mechanical herbivory defenses, and seed dispersal mechanisms (Abbo et al., 2022; Pickersgill, 2007). Domestication breeds out evolutionarily established fitness mechanisms if the trait is not specifically desired by humans (Abbo et al., 2022). One of these mechanisms is the arbuscular mycorrhizal (AM) symbiosis which has coevolved with plants over a long evolutionary history as a beneficial symbiont, and indeed domestication has sometimes damaged this relationship (Hetrick et al., 1992).

Following WWII, the modern age of agriculture ushered in the widespread use of inorganic fertilizer (Ashley et al., 2011). Cultivars bred under high nutrient conditions no longer relied on the AM symbiosis to acquire sufficient nutrients. This was clearly observed in a study by Hetrick et al. (1995) that showed that wheat varieties released before 1975 displayed higher mycorrhizal response compared to modern cultivars. Maintenance of mutualisms is costly if their benefit is gained in an easier way, and the mass application of inorganic fertilizer gave plants the benefit of an abundance of nutrients without the cost of 'feeding' the fungi (Pérez-Jaramillo et al., 2015).

Additionally, the loss of soil microbial diversity in agricultural systems may have interfered with the beneficial interaction between plants and AMF and made the AMF less accessible to the cultivated plants, breaking up this mutualism (Pérez-Jaramillo et al., 2015). Studies have found that modern cultivars have a decreased ability to sustain the AM symbiosis, as seen in breadfruits in which modern cultivars had reduced colonization compared to wild ancestors (Xing et al., 2012).

Although modern breeding has developed cultivars that are optimized to have the greatest economic output and best adapted to agricultural systems, this may have led to a lower stress tolerance, leaving them vulnerable to small changes to their environment (Pérez-Jaramillo et al., 2015). For example, domesticated plants are adapted to local climates, and lack defenses against herbivory and pathogens (Abbo et al., 2022; Chen et al., 2015; Xing et al., 2012). With threats of climate change each of these aspects are expected to be major stressors on global crops. Because AMF are known to alleviate biotic and abiotic stresses such as pathogens and drought, the application of AMF on highly domesticated crops may incur a substantial benefit (Begum et al., 2019; Lee et al., 2013; Smith and Smith, 2012).

Overall, modern breeding has caused plants to become less dependent on the mycorrhizal symbiosis; therefore, older cultivars tend to be more sensitive to AMF inoculation (Kokkoris et al., 2019; Xing et al., 2012). As such, it is crucial to explore AMF traits using both modern and old cultivars in order to reveal effects that may be indistinct in modern cultivars.

1.7 *Solanum tuberosum* L.

Solanum tuberosum L. (potato) is a tuber crop in the family Solanaceae (Jansky and Spooner, 2017). Potatoes are recognized as the third most important food crop and a major player in addressing food security as they are grown globally and produce large quantity of food in small areas of land (Jansky and Spooner, 2017). As well, potatoes are high in nutrition (e.g., protein, fibre, vitamin C, antioxidants; Camire et al., 2009; Jansky and Spooner, 2017; Kolasa, 1993; Navarre et al., 2009).

Potatoes originated from two groups in South America, the Andigenum group from the High Andes and the Chilotanum group from Chile, and slowly spread throughout the world, becoming widely used in Europe beginning in the late 1700s. The crop was bred in Europe and North America in the mid 1800s (Jansky and Spooner, 2017). The domestication of potatoes selected for larger tubers, various colours and shapes of tubers, as well as the reduction of bitter glycoalkaloids (Spooner et al., 2005).

Potatoes have mostly been bred through clonal propagation, creating a deficiency of fertile males, making it difficult to breed sexually, and rid the cultivars of deleterious mutations (Jansky and Spooner, 2017). In 1933, following the implementation of national breeding efforts in the United States of America, in response to the prevalence of potato viruses, true seeds were produced, and hybridization of cultivars began, marking the beginning of modern breeding in the US (Jansky and Spooner, 2017).

Potatoes have high fertilizer demands (Davies et al., 2005; Wu et al., 2013), requiring a large amount of P and N for optimum growth and tuber yield (Haase et al., 2007; Mokrani et al., 2018; Nitsos and Evans, 1969; Poljak et al., 2007). Unused photosynthates are transferred into the tubers which are used as storage organs for the potato (Katoh et al., 2015). P and N stress can limit photosynthesis and negatively influence partitioning of photoassimilates (e.g., NADPH, and ATP) and photosynthates (e.g., carbon) from leaves to tubers leading to smaller tubers (Katoh et al., 2015; Mokrani et al., 2018). As well, deficiency in N in potatoes leads to a higher level of carbon allocated to the roots, meaning an increased root:shoot ratio (Mokrani et al., 2018).

Regardless of the high nutrient demand, potatoes have shallow root systems and sparse root hairs making them inefficient at absorbing soil nutrients (Wu et al., 2013). Because of these characteristics, potatoes respond well to the mycorrhizal symbiosis (Hijri, 2016). Under various experiments, potatoes have been found to benefit from mycorrhizal inoculation, increasing shoot and root biomass (Graham et al., 1976; Wu et al., 2013) and increasing crop yield (Black and Tinker, 1977; Hijri, 2016), making potatoes an ideal host organism for AMF research.

1.8 Research Justification and Goals

Based on evidence from *in vitro* AMF research, and knowledge from relatives in the phyla Ascomycota and Basidiomycota, the dikaryotic organization presents a nuclear condition that may cause distinct phenotypic responses compared to homokaryotic relatives leading to distinct responses in AMF host plants. Due to increased genetic diversity and the ability to adjust nucleotype abundance in dikaryotic strains, I predict that mycorrhizal response will be positively higher when plants are inoculated with dikaryotic strains compared to homokaryotic strains. Mycorrhizal responses may differ based on the identity of the host plant and its breeding history. Because modern breeding has led plants to be less dependent on the AM symbiosis, modern cultivars may experience less significant distinctions in responses between these two genetic organizations. Therefore, I also predict that differences in mycorrhizal response between dikaryotic and homokaryotic strains will be higher in older cultivars compared to modern cultivars. To test these predictions, four dikaryotic and four homokaryotic AMF strains have been applied to four cultivars (two old and two modern) of *Solanum tuberosum* in a greenhouse experiment to determine the impact of genetic organization on the mycorrhizal response of modern and old cultivars of this species.

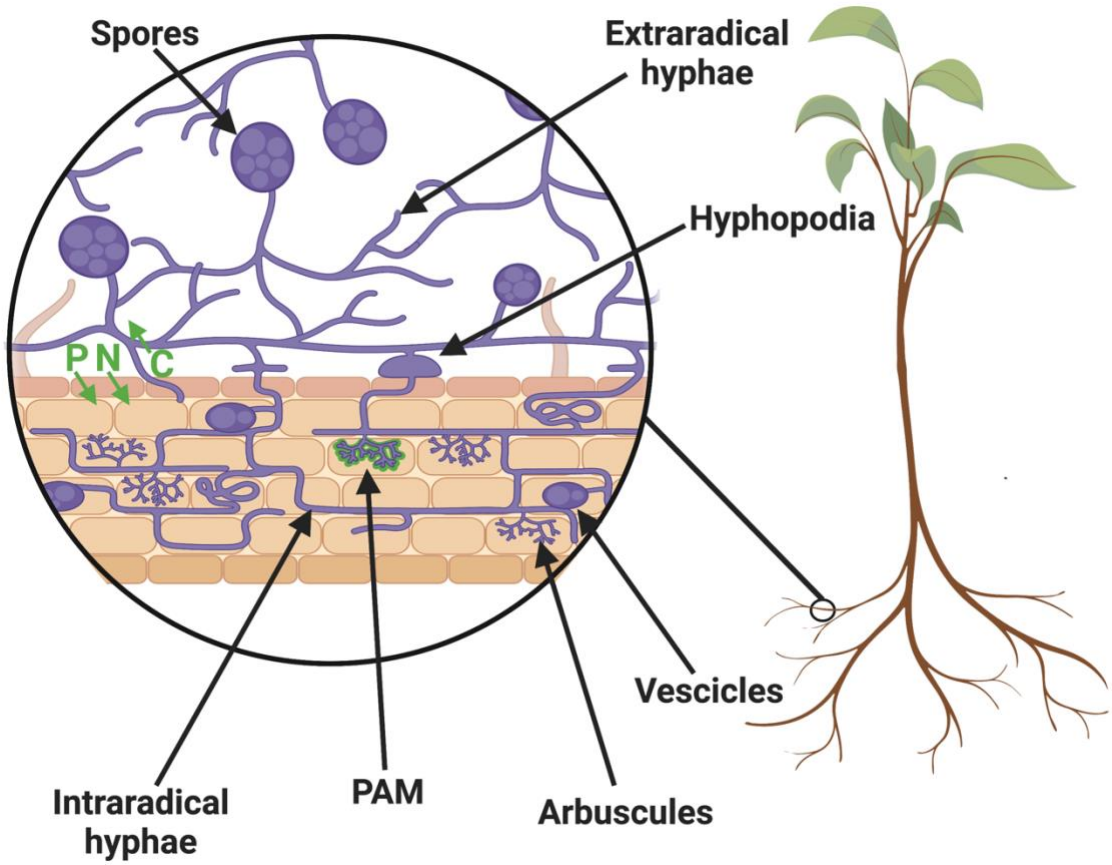


Figure 1.1. Arbuscular mycorrhizal fungi colonizing a plant root. The mycelium of AMF is made up of branching tube-like filaments called hyphae. Extraradical hyphae spread an extensive network throughout the soil microbiome (extraradical hyphae) and form storage organs called spores which can be used as propagules. Hyphae form adhesion structures (hyphopodia) on the root epidermis. Hyphae penetrate the roots (intraradical hyphae) extending both intercellularly and intracellularly. Here, they form vesicles which, similarly to spores, store lipids and may act as propagules. In the cortex of the root the hyphae enter single cells where they form highly branched structures called arbuscules. Plant derived peri-arbuscular membrane (PAM) surrounds the arbuscule and together, nutrients (Phosphorus, Nitrogen, Carbon, and others) are exchanged between AMF and host. Created with biorender.com.

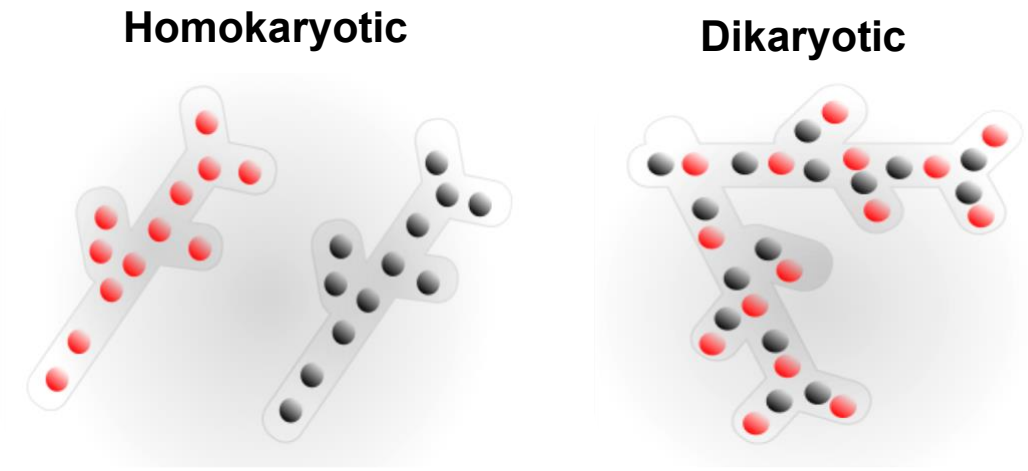


Figure 1.2. In the model AMF species, *R. irregularis*, strains are either dikaryon-like (or AMF dikaryons); or homokaryons. AMF homokaryons contain hundreds of nuclei of one genotype. AMF dikaryons contain hundreds of nuclei of two divergent genotypes (nucleotypes) from two parental strains. Within AMF dikaryons, the two nucleotypes contain divergent genomic regions that are similar to the mating type (MAT) loci, which determine sexual compatibility in closely related fungal phyla, Ascomycota and Basidiomycota. Red circles represent nucleotype MAT-A, Black circles represent a divergent nucleotype MAT- B. Figure modified from Ropars et al. (2016).

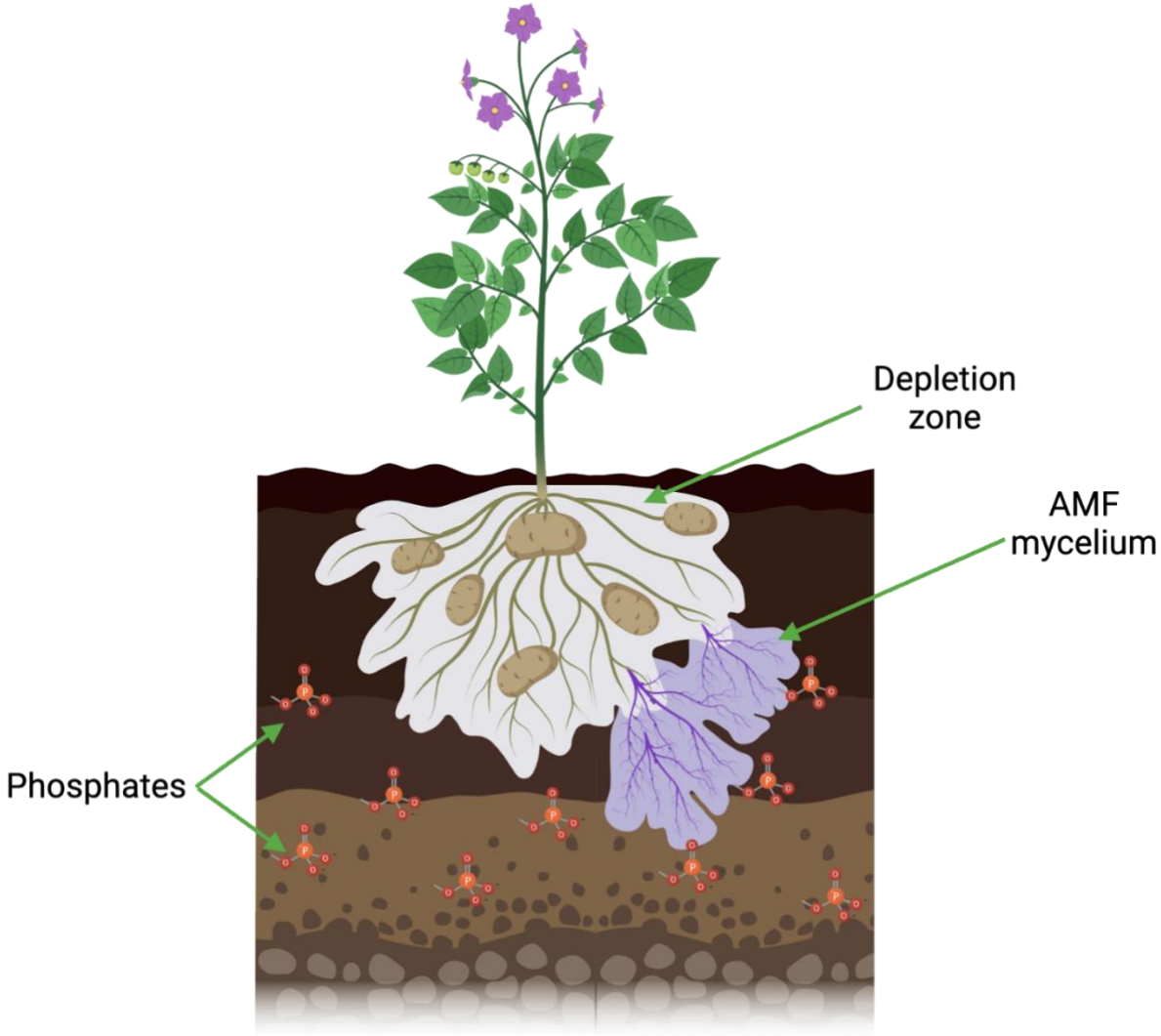


Figure 1.3. AMF extend the area of nutrient access beyond the depletion zone of the soil surrounding plant roots. Once a plant has absorbed the nutrients within the reach of the roots, the plant is then limited by these depleted nutrients, particularly nutrients which are immobile in the soil, like phosphates. AMF act as an extension of the root system, capable of accessing nutrients in tiny crevices and well beyond the depletion zone. The AMF transport nutrients from a much greater surface area to the plant, increasing the plant's nutrient absorption by more than 100 fold. Created with biorender.com.

CHAPTER TWO

Manuscript:
Mycorrhizal response of homokaryotic versus dikaryotic arbuscular mycorrhizal fungi.

2.1 Abstract

Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts with a unique nuclear organization in which thousands of nuclei co-exist. These nuclei are either genetically similar (homokaryotic) or derived from two distinct parents (dikaryotic). We investigated the impact of these two genetic organizations on the AMF symbiosis by measuring the mycorrhizal response (MR) of potato cultivars with different degrees of domestication using representative AMF homokaryotic and dikaryotic strains. The genetic organization (dikaryotic vs homokaryotic) and domestication status (old vs modern cultivars) of the host are both significant factors in the MR of host plants. Specifically, biomass is significantly greater when inoculated with homokaryotic AMF compared to dikaryotic AMF. Arbuscule colonization shifts depending on host domestication status in the presence of AMF dikaryons, but not with homokaryotic strains. Consistent with past research, nutrient (N and P) uptake is greater in old cultivars than modern cultivars. Analyses of single spores from AMF dikaryons using digital droplet PCR (ddPCR) confirms that the nucleotype ratio of these strains shifts depending on host identity. Together, these reveal significant differences in MR between old and modern cultivars after inoculation with homokaryotic and dikaryotic AMF under greenhouse conditions.

2.2 Keywords

Mycorrhizal response, AMF dikaryons, mycorrhiza, domestication, potato

2.3 Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts that colonize the roots of approximately 80% of all vascular land plants (Smith and Read, 2008) including most economically important agricultural crops. There are approximately 327 AMF species described to date (Błaszkowski et al., 2022; "International Culture Collection of Glomeromycota [CIGC]," 2022; Stürmer and Kemmelmeier, 2021), each with the ability to form mycorrhizas with a diverse array of plant hosts, often even simultaneously with multiple hosts. The origin of the arbuscular mycorrhizal (AM) symbiosis likely dates back at least 400 million years (Bonfante and Selosse, 2010; Selosse and Tacon, 1998; Wang and Qiu, 2006).

AMF display a dual niche, growing simultaneously inside plant roots (intraradical mycelium) and in the surrounding soil where they spread an extensive hyphal network (extraradical mycelium; Brachmann and Parniske, 2006; Selosse and Tacon, 1998). The extraradical mycelium transfers soil nutrients such as phosphorous and nitrogen into the roots of their host plants (Smith and Smith, 2012). Subsequently, in specialized structures of the intraradical mycelium known as arbuscules, the fungi exchange these nutrients for carbon, in the form of lipids and sugars (Keymer et al., 2017; Smith and Smith, 2012). Besides the increased nutrient uptake that AMF provide to their hosts, they can also provide an array of other important benefits, including improved plant tolerance to both biotic and abiotic stressors such as drought, toxins, and disease (M. Chen et al., 2018; Jung et al., 2012b; Liu et al., 2018; Mitra et al., 2022). Because of these benefits and of their generalist nature, AMF have increasingly been used and researched in the agriculture industry in the last three decades (Berruti et al., 2016; Gianinazzi et al., 2010; Hamel, 1996) promising a reduction of chemical fertilizer input while promoting efficient crop yields (Ceballos et al., 2013; Liu et al., 2018).

An important measure that defines the mutualistic quality of AMF is known as mycorrhizal response (MR). MR describes any benefit or detriment that the plant exhibits, for a specific trait (e.g. nutrient uptake, biomass etc.), as a consequence of inoculation with AMF (Baon et al., 1993; Sawers et al., 2008). MR is highly dependent on the plant and fungal identity (Dai et al., 2014; Ryan and Kirkegaard, 2012) with fungal species exhibiting higher intraspecific than interspecific variation (Hart and Reader, 2002; Stahl and Christensen, 1991). While the symbiosis is at large mutualistic, negative and neutral MR is often observed (Ceballos et al., 2019; Klironomos et al., 2000; Ryan et al., 2005).

Although the high variation in MR among AMF strains suggests a single strain could not be optimal for all plant species, the model species *Rhizophagus irregularis* (specifically the strain DAOM-197198) is the main source of inoculum across studies and commercial products, however there are discrepancies in its effectiveness (Stockinger et al., 2009). For example, some studies report a high positive MR, as seen in potatoes (increased yield and increased tuber size; (Hijri, 2016) while others report negligible responses as seen in peas (Jin et al., 2013). There are even negative responses to inoculation with the commercialized strain DAOM-197198, with several studies observing growth depression as seen for example in barley (Christophersen et al., 2009; Grace et al., 2009).

A clear cause for the inconsistencies observed in the MR is yet to be determined. To investigate this, it is important to understand the unique biology of AMF. AMF contain hundreds to thousands of nuclei in each spore and throughout their unsegmented hyphae (Kokkoris et al., 2020). In *R. irregularis*, strains are found to be either dikaryon-like (or AMF dikaryons); containing hundreds of nuclei of two divergent genotypes (nucleotypes) from two parental strains, or homokaryotic; containing hundreds of nuclei of one genotype (E. C. H. Chen et al., 2018; Ropars et al., 2016). The commercial strain DAOM-197198 belongs to the second category

(homokaryotic) along with the majority of available *R. irregularis* strains. Interestingly, only five (out of 114) strains tested for this species have so far been found to be dikaryotic (Kokkoris et al., 2021).

In a recent experiment, the relative abundance of each nucleotype of four dikaryotic strains of *R. irregularis* was examined using droplet digital PCR. It was found that these strains have the potential to regulate the abundance of each nucleotype in response to plant host identity (Kokkoris et al., 2021). Another experiment revealed that *R. irregularis* dikaryons have distinct life history strategies compared to their homokaryotic relatives, including faster growth and the ability to produce more complex hyphal networks compared to homokaryons, which may result in higher rates of germination and better ability to colonize multiple plants (Serghi et al., 2021). These findings suggest that AMF dikaryons may have an adaptive advantage in terrestrial ecosystems. The distinct life history strategy and regulation of the nucleotype ratios could affect the plant host response through differentiated protein expression of both partners, altering the function of the fungus, as seen in other major fungal groups.

The heterokaryotic (containing two or more genetically distinct nuclei) strains of the closely related fungal phyla Basidiomycota and Ascomycota also contain two parental nucleotypes, and this genetic condition can positively affect the phenotype and fitness of these strains (Clark and Anderson, 2004; Parmeter et al., 1963; Strom and Bushley, 2016). In some cases, dikaryons created by genetically divergent parents express novel gene combinations that allow the hybrids to perform better than either of the parental strains (Beadle and Coonradt, 1944; Strom and Bushley, 2016). Consistent with recent data from AMF (Kokkoris et al., 2021), heterokaryons of other fungal groups can regulate their nucleotype ratios in response to their environment and their host, allowing them to adjust their phenotypic and functional traits to each condition (James et al., 2008; Jinks, 1952). Co-existing nuclei can also interact by masking a

deleterious allele in one nucleus with a dominant allele in the neighbouring nucleus (Amburgey, 1970; Clark and Anderson, 2004), or by complementary effects of the two nuclei (Wagner et al., 1988) generated by both nuclei being simultaneously expressed (Ingold and Hudson, 1993). The heterokaryotic advantage enabled by these unique nuclear dynamics is also reflected in the fungi's interaction with symbiotic hosts. For example, in ectomycorrhizal fungi additive effects in the dikaryotic stage lead to increased nitrogen uptake in host plants (Wagner et al., 1988). In summary, ascomycetes and basidiomycetes benefit from the co-existence of multiple genotypes in their cells, as demonstrated repeatedly over nearly 100 years of research on this topic (Beadle and Coonradt, 1944; James et al., 2008; Parmeter et al., 1963; Samils et al., 2014; Strom and Bushley, 2016).

Recently, the potential benefit on MR of dikaryotic over homokaryotic AMF strains was studied in a greenhouse study on cassava plants. Peña et al., (2020) found that the recovery rate of plants inoculated with a dikaryotic strain of *R. irregularis* (C3) was significantly higher than that of a homokaryon strain (A1) of the same species, and the plant inoculated with the dikaryotic strain was somewhat less susceptible to drought. These findings provide initial evidence that genetic organization may have an important role in the functioning of AMF as a symbiont. However, whether similar effects are found in different hosts and conditions is not known. Furthermore, wild plants have been observed to be more sensitive to AMF inoculation (Kokkoris et al., 2019; Xing et al., 2012) compared to modern cultivars (Hetrick et al., 1992; Sawers et al., 2008; Xing et al., 2012; Zhu et al., 2001), but how MR of dikaryotic vs homokaryotic strains varies among cultivars has never been studied.

In this study I aim to determine the impact of AMF genetic organization (dikaryotic versus homokaryotic strains) on the MR of a highly mycorrhizal responsive plant (potatoes; *Solanum tuberosum* L.) using old and modern cultivars. In doing so I hypothesize that:

a) MR will be greater when hosts are inoculated with dikaryotic AMF strains compared to homokaryotic strains due to the increased genetic diversity present and the ability to adjust the relative nucleotype abundance in dikaryotic strains.

b) Differences in MR between plants inoculated with dikaryotic versus homokaryotic strains will be greater in old cultivars compared to modern cultivars due to the low responsiveness of modern cultivars to AMF.

To assess these hypotheses, I completed an *in plantae* greenhouse experiment to determine the MR of old and modern cultivars of potato plants to inoculation with various homokaryotic and dikaryotic strains of *R. irregularis*.

2.4 Materials and Methods

2.4.1 Experimental treatments

We performed a greenhouse experiment to test the effect of genetic organization (homokaryotic and dikaryotic strains) on MR of old and modern potato cultivars (*Solanum tuberosum* L.). We had 32 treatments consisting of a combination of four potato cultivars and eight AMF strains as well as an uninoculated control treatment for each potato cultivar. Each treatment had six replicates (total 216 experimental units). Plants that died during the growth period were removed from the experiment. At the end of the experiment each treatment had a minimum of 4 replicates. The experiment was conducted from February to June 2021 in the greenhouse at the University of Ottawa's Center for Advanced Research in Environmental Genomics.

2.4.2 Inoculant identity

All strains used were of the species *Rhizophagus irregularis*, a model AMF species that has all of the five known AMF dikaryotic strains (Kokkoris et al., 2021; Ropars et al., 2016). We used dikaryotic strains A4 (MAT1 & MAT2), A5 (MAT6 & MAT3), SL1 (MAT1 & MAT5), and G1 (MAT1 & MAT5) and homokaryotic strains 330 (MAT2), 66 (MAT3), 101 (MAT5), and C2 (MAT6) that were selected based on genetic similarities of the mating type (MAT) locus of the dikaryotic strains (Figure S1). Each strain was acquired from pre-existing cultures in the Corradi Lab inventory.

Each strain was propagated using Agrobacterium root-inducing (Ri) T-DNA transformed root organ cultures of *Daucus carota* (cultivar P68), growing in double compartment petri dishes with M medium as described by Bécard and Fortin, (1988). Using dual compartment dishes allowed for the development of pure fungal inoculum that was easily extractable. When the AMF had grown and sufficiently sporulated on the opposite side of the dish (approximately 3 months), spores were extracted and homogenized using sodium citrate buffer to dissolve the media (Doner and Bécard, 1991). Approximately 50 (+/-3) spores were inserted into a petri dish of agar to form inoculation pucks to avoid spore runoff in the pots.

2.4.3 Plant Identity

Potatoes (*Solanum tuberosum* L.) have shown to be highly responsive to the AM symbiosis (Black and Tinker, 1977; Davies et al., 2005; Hijri, 2016; McArthur and Knowles, 1993). Due to their sparse root hairs and shallow root systems, potatoes are inefficient at absorbing phosphorus (Liu et al., 2018; Yamaguchi, 2002) but AMF mycelium acts as an extension of the root system, compensating for the potato's shortcomings (Smith and Read, 2008). Additionally, potatoes are a

key crop that heavily relies on fertilizer input (Hijri, 2016), making them an ideal host to observe the effect of AMF genetic organization. The potato varieties were provided by Agriculture Canada. These plants were propagated at Agriculture Canada and received as *in vitro* plantlets. We used four varieties, two old cultivars (established pre-1935) and two modern varieties; Table 1).

Table 2.1. *Solanum tuberosum* varieties.

Variety	Year Established	Tuber shape	Tuber skin
Slovenian crescent	Heritage/ unknown	Fingerling	Buff
Katahdin	1933	Elliptical	Buff
Red gold	1970	Round-Oval	Pinkish Red
AC Belmont	1967	Round-Oval	White

2.4.4 Inoculation and growing conditions

We filled 2.84 L plastic pots with sterilized medium composed of 75% Appalache Valley All Purpose Sand and 25% Holiday Vermiculite by volume. We placed inoculum pucks in the soil, followed by the potato plantlets and soil to fill the pots. Control pots had agar pucks with no spores placed in them. The plants were watered with a dripping irrigation system at a rate of 2nL per hour for one minute (35 mL) daily; after 50 days the same regimen was applied twice per day.

The soil medium had no nutrients, and a low phosphorus environment was maintained by using a low phosphorus fertilizer (ICL Peter Excel 15-5-15 Cal-Mag special). 15 mL of fertilizer was diluted in 4 L of water and 50 mL of solution (29.1 mg N and 4.24 mg P) was applied to each pot every 2 weeks.

The pots were arranged in a completely randomized block design, with 6 blocks (one for each replicate) to account for the environmental variability of the greenhouse. An additional pot for each treatment was made in order to confirm AMF colonization. Colonization was confirmed one month into the experiment.

16 hours of light per day was applied to the plants. The temperature in the greenhouse ranged from 18 to 35 ˚C.

2.4.5 Mycorrhizal responses (MR)

We evaluated any benefit or detriment that the plants received from the AMF inoculation as mycorrhizal responsiveness (MR) using the following formula: MR = ln (A/B) where A = response of one mycorrhizal plant and B = mean response of all non-mycorrhizal plants from the corresponding potato variety. We evaluated the MR for the total plant biomass, root: shoot ratio, tuber biomass, tuber number, and percent nutrient content for shoots and tubers (specifically nitrogen and phosphorus).

During harvest, the shoots, roots, and tubers were separated and weighed, and tubers were counted. Following harvest, each was dried in a 70 ˚C drying oven and weighed every 3 days until mass from one measurement to the next was unchanged. Then, final mass was measured. Dry roots and shoots were used to determine root:shoot ratio (root mass/shoot mass).

Dry tubers and shoots were ground using a coffee grinder (Proctor Silex®Fresh Grind™) and samples were sent to Agriculture Canada where percent nitrogen and phosphorus in the shoots and tubers were analyzed. The samples were digested using the Kjeldahl method (Nelson and Sommers, 1973) and % nitrogen and phosphorus were assessed using a Flow Injection Analysis Auto-analyzer.

2.4.6 Fungal responses

During harvest, fresh roots were cut into 2 cm segments, homogenized, and approximately 0.5-1g of root was placed in cassettes and stored in distilled water. The following day, roots were stained using the ink-vinegar method (Vierheilig et al., 1998) using black Sheaffer Skrip ink. Ten to twelve root fragments were then placed on 3 glass slides per pot (minimum of 30 root fragments total per pot). The percentage of root colonization and fungal organs (vesicles and arbuscules) were assessed microscopically and quantified using the Trouvelot method (Trouvelot, 1986). All uncolonized plants were removed from the experiment.

To assess extraradical colonization we measured external spore abundance using the spore extraction protocol by Gerdemann and Nicolson (1963). We first collected 70 mL of soil from each pot, blended it for 5 seconds, and filtered it through multiple sieves to catch the soil and release the spores into a smaller sieve 63µm in size. The sample was then collected in falcon tubes and centrifuged twice (at 1200 RPM and 960 RPM).

2.4.7 Nucleotype abundance

We extracted 20 spores from the soil of each dikaryotic treatment using the above-mentioned method. We assessed the relative nucleotype abundance using droplet digital PCR (ddPCR) with the method described in Kokkoris et al. (2021). We isolated single spores, washed them in autoclaved distilled water, and placed them in 0.2 mL PCR tubes using a pipette under a stereoscope and added 2.4 µL of autoclaved distilled water. We then crushed the spores using a sterilized needle under stereoscope, to release the nuclei. We then added master mix of 1X Supermix for Probes (Bio-Rad), 1 mL of 500 nM primers to 250 nM probe mixture (PrimeTime std qPCR Assay (500rxn)), and DNase free water to the reaction tube. Then droplets were

generated from this mixture using a QX100™ droplet generator to split the sample into approximately 20000 droplet compartments. The sample was then amplified using a C1000 Touch Thermal Cycler (Bio-Rad Technologies, Inc, Mississauga, ON, Canada). Cycling conditions for the PCR were as described in Kokkoris et al. (2021). Droplet data was analyzed using the Quantasoft™ Analysis Pro (1.0.596; Bio-Rad) software and assessed as either Nucleotype A (HEX dye probe fluorescing green), Nucleotype B (FAM dye probe fluorescing blue) or no signal (Figure S2). Primer/probes were designed based on the mating-type loci of each nucleotype by Kokkoris et al. (2021). FAM probes were used to label one nucleotype per strain and Hex probes were used to label the other nucleotype.

2.4.8 Statistical analysis

We used a permutational multivariate analysis of variance (PERMANOVA) to determine whether the MR of dikaryotic inoculation and homokaryotic inoculation differed as well as to determine whether the MR of modern and old cultivars differed. The PERMANOVA was performed by using the function, Adonis (package "Vegan" version 2.5-7; Oksanen et al., 2020). All measured MR were used as response variables, excluding root biomass since its correlation coefficient with other responses exceeded 0.8. Genetic organization (homokaryon vs. dikaryon) and domestication (modern vs. old) were fixed factors and strain identity, block, and cultivar were random factors. The PERMANOVA used Euclidian distance metric as dissimilarity index and 999 permutations. Data was standardized using the "normalize" method and the "decostand" command in R (package "Vegan" version 2.5-7; Oksanen et al., 2020) prior to the PERMANOVA. The results were visualized with a non-metric multidimensional scaling (NMDS) plot using the package ggplot2 (Wickham, 2016) in R studio.

To examine individual traits (total biomass, tuber number, % shoot P etc.) differences between dikaryons and homokaryons and between modern and old cultivars, we used linear mixed effect model (package "lme4" version 1.1-28; Bates et al., 2015) with genetic organization (dikaryon vs. homokaryon) and plant provenance (modern vs old) as fixed factors, and inoculum strain, cultivar, and block as random factors.

To determine if the relative nucleotype abundance within dikaryotic spores differed significantly depending on cultivar, we used a Kruskal-Wallis Test (package "stats" version 4.1.0; R Core Team, 2021) due to the non-normal distribution of our data despite transformations, as well as variation in sample size between the groups. We used pairwise Wilcoxon signed rank test to perform pairwise comparisons between cultivars following the Kruskal-Wallis analysis.

To determine if the abundance of spores in the soil differed significantly between dikaryotic and homokaryotic AMF and between modern and old cultivars, we used a negative binomial generalized mixed effects model (package "lme4" version 1.1-28; Bates et al., 2015) due to the non-normal distribution of residuals despite transformations and overdispersion of data. Fixed factors were genetic organization (dikaryon vs. homokaryon) and plant provenance (modern vs old), and random factors were inoculum strain, cultivar. Block was not used as a random effect as its variance was zero.

To determine if colonization differed between dikaryotic and homokaryotic AMF and between modern and old cultivars we used a linear mixed effect model (package "lme4" version 1.1-28; Bates et al., 2015) with genetic organization (dikaryon vs. homokaryon) and plant provenance (modern vs old) as fixed factors, and inoculum strain, cultivar, and block as random factors. % Colonization was normalized using ordered quantile normalizing transformation (package "bestNormalize" version 1.8.3; Peterson, 2021) to allow for normal distribution of

residuals in the model. To assess abundance of fungal organs (% arbuscule and % vesicles) we used a negative binomial generalized mixed effect model (package "lme4" version 1.1-28; Bates et al., 2015) as normalization of residuals was not possible and the data was over dispersed. Fixed and random factors were as above.

R studio (Version 1.4.1717 – © 2009-2021 RStudio, PBC) was used for all statistical analyses (R Core Team, 2021).

2.5 Results

2.5.1 MR of homokaryotic vs dikaryotic AMF inoculation

In agreement with our hypothesis, there was a significant difference in MR between plants inoculated with homokaryotic and dikaryotic AMF ($p < 0.001$; fig. 2.1).

There was a significant interaction between AMF genetic organization (homokaryotic vs dikaryotic) and plant domestication (old vs modern) such that when inoculated with homokaryotic AMF modern cultivars have significantly lower shoot biomass response (interaction; $p < 0.05$; fig.2.2d) than old cultivars, as opposed to dikaryotic inoculation which shows an insignificant response between old and modern cultivars. Plants inoculated with homokaryotic AMF had greater tuber biomass ($p < 0.01$), total biomass ($p < 0.01$), and number of tubers ($p < 0.01$) compared to dikaryotic AMF (fig. 2.2). There was a near significant difference between homokaryotic and dikaryotic inoculation in % shoot nitrogen ($p = 0.05$; fig. 2.2e) such that dikaryotic treatments have somewhat greater % shoot nitrogen. There was not a significant difference between homokaryotic and dikaryotic inoculation in shoot biomass ($p = 0.06$), root:shoot ratio ($p = 0.21$), % tuber nitrogen ($p = 0.45$), % tuber phosphorus ($p = 0.10$), or % shoot phosphorus ($p = 0.38$; Table S1).

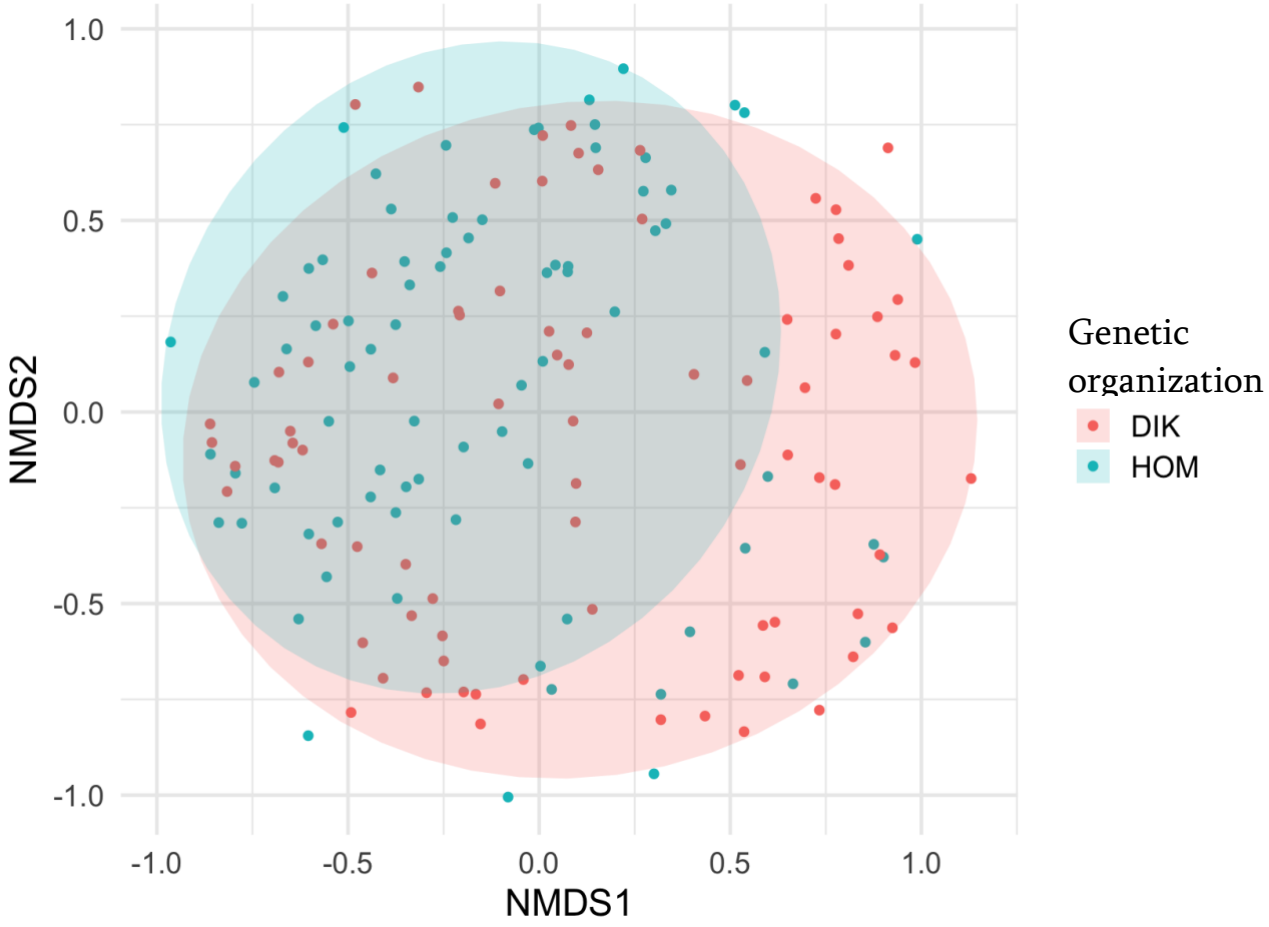


Figure 2.1. Euclidian distance based non-metric multidimensional scaling (NMDS) plot of potato plants inoculated with homokaryotic (blue dots) and dikaryotic (red dots) AMF strains. Stress = 0.24. Ellipses represent an 85% confidence intervals around homokaryotic (blue) and dikaryotic (red) treatments.

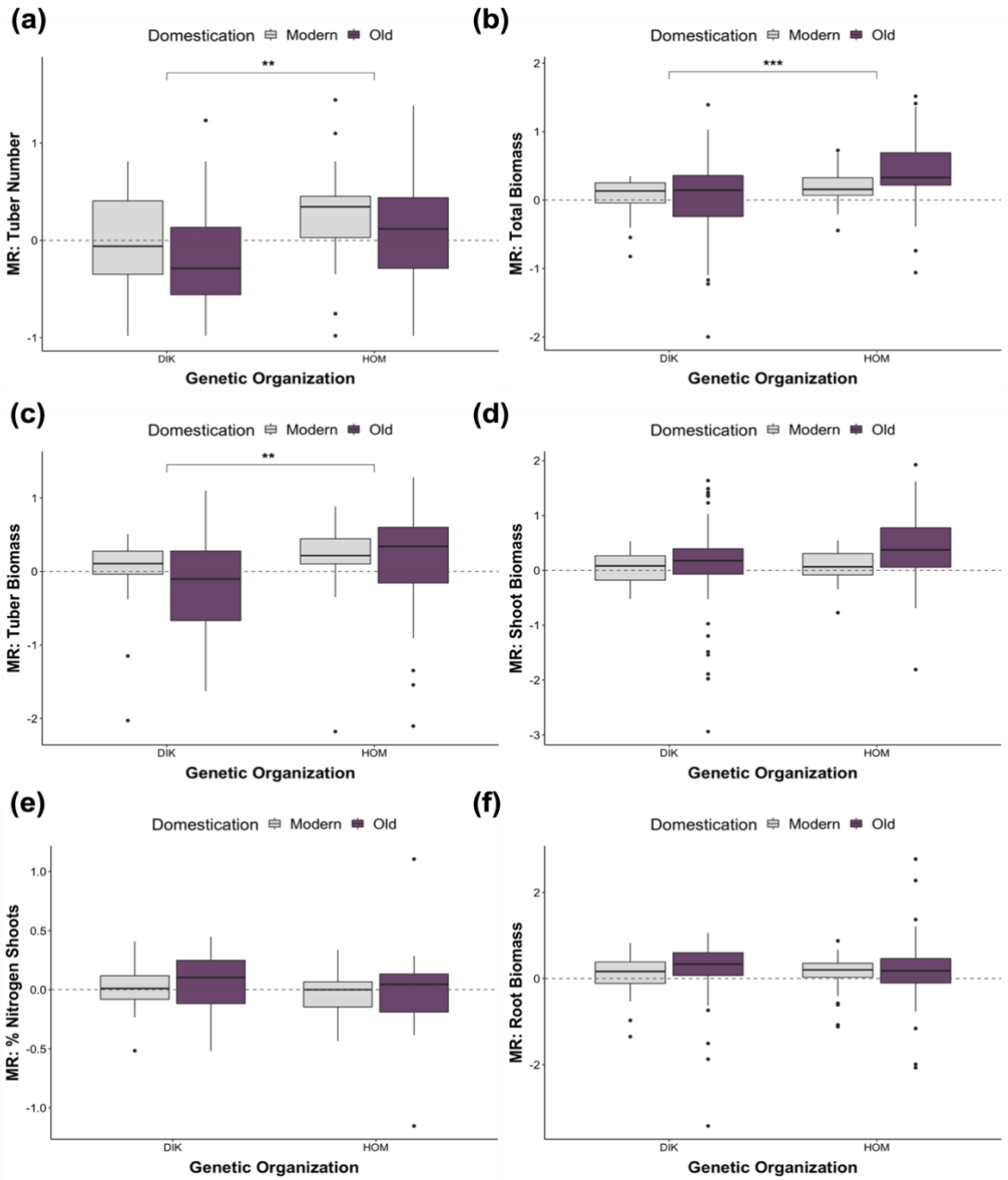


Figure 2.2. Mycorrhizal response of potato plants inoculated with homokaryotic and dikaryotic strains of AMF. (a) tuber number, (b) total biomass, (c) tuber biomass, (d) shoot biomass, (e) percent tuber nitrogen, (f) root biomass. Dotted line indicates the value of the uninoculated control plants. Values above the dotted line indicate greater than the control and values below the dotted line indicated less than the control. Mycorrhizal response (MR) is calculated as MR = ln (A/B) where A = response of individual mycorrhizal plants and B = mean response of non-mycorrhizal plants. Boxplots show the third quartile and first quartile (box edges), median (middle line), the range of the data (whiskers), and data outliers (black dots). Modern-DIK n = 43, modern-HOM n = 35, old-DIK n = 39, old-HOM n = 44. Asterisks indicate a significant difference between the MR of homokaryotic treatments and dikaryotic treatments. * signifies $p<0.05$, ** signifies $p<0.01$, *** signifies $p<0.001$.

2.5.2 MR of modern versus old cultivars

In agreement to our hypothesis, there was a significant difference in MR between old cultivars and modern cultivars ($p < 0.001$; fig.2.3).

There was a significant interaction between AMF genetic organization (homokaryotic vs dikaryotic) and plant domestication (old vs modern) such that modern cultivars have significantly less % tuber P than old cultivars when inoculated with homokaryotic AMF (interaction $p<0.01$; fig.2.4c). Plant provenance also had a significant impact on % tuber P such that the old cultivars had significantly increased % tuber P ($p < 0.001$), % shoot P ($p < 0.01$), and % tuber N ($p < 0.001$) compared to modern cultivars (fig.2.4). Plant provenance also had a significant impact on the root:shoot ratio such that the modern cultivars had significantly increased root:shoot ratio compared to old cultivars ($p<0.001$). Domestication did not have a significant impact on total biomass ($p = 0.27$), shoot biomass ($p = 0.14$), tuber biomass ($p = 0.49$), or tuber number ($p = 0.45$; Table S1).

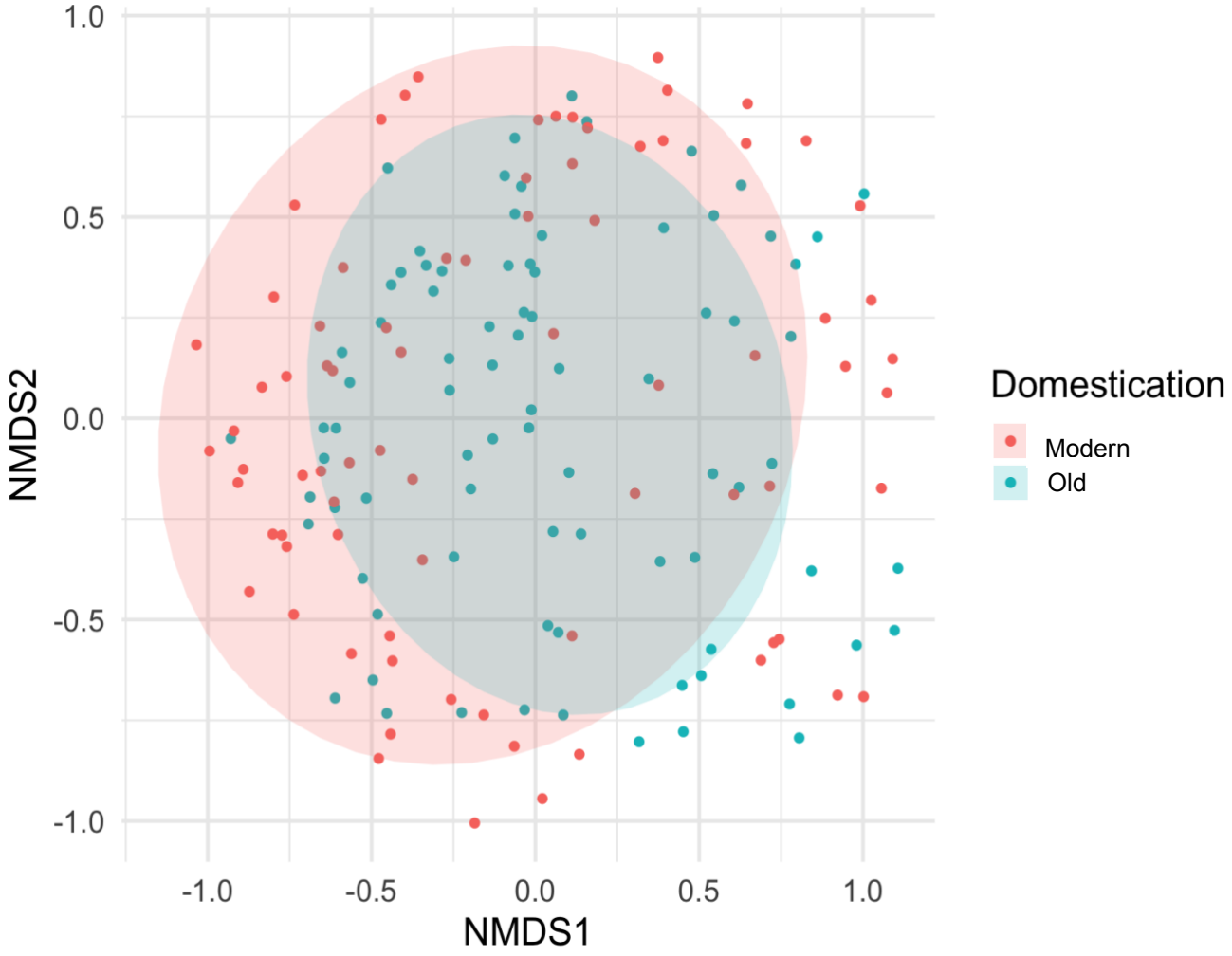


Figure 2.3. Euclidian distance based non-metric multidimensional scaling (NMDS) plot of modern (red dots) and old (blue dots) cultivars based on the measured mycorrhizal response of four potato cultivars inoculated with eight strains of *R. irregularis*. Stress = 0.24. Ellipses represent an 85% confidence intervals around modern (red) and old (blue) cultivars.

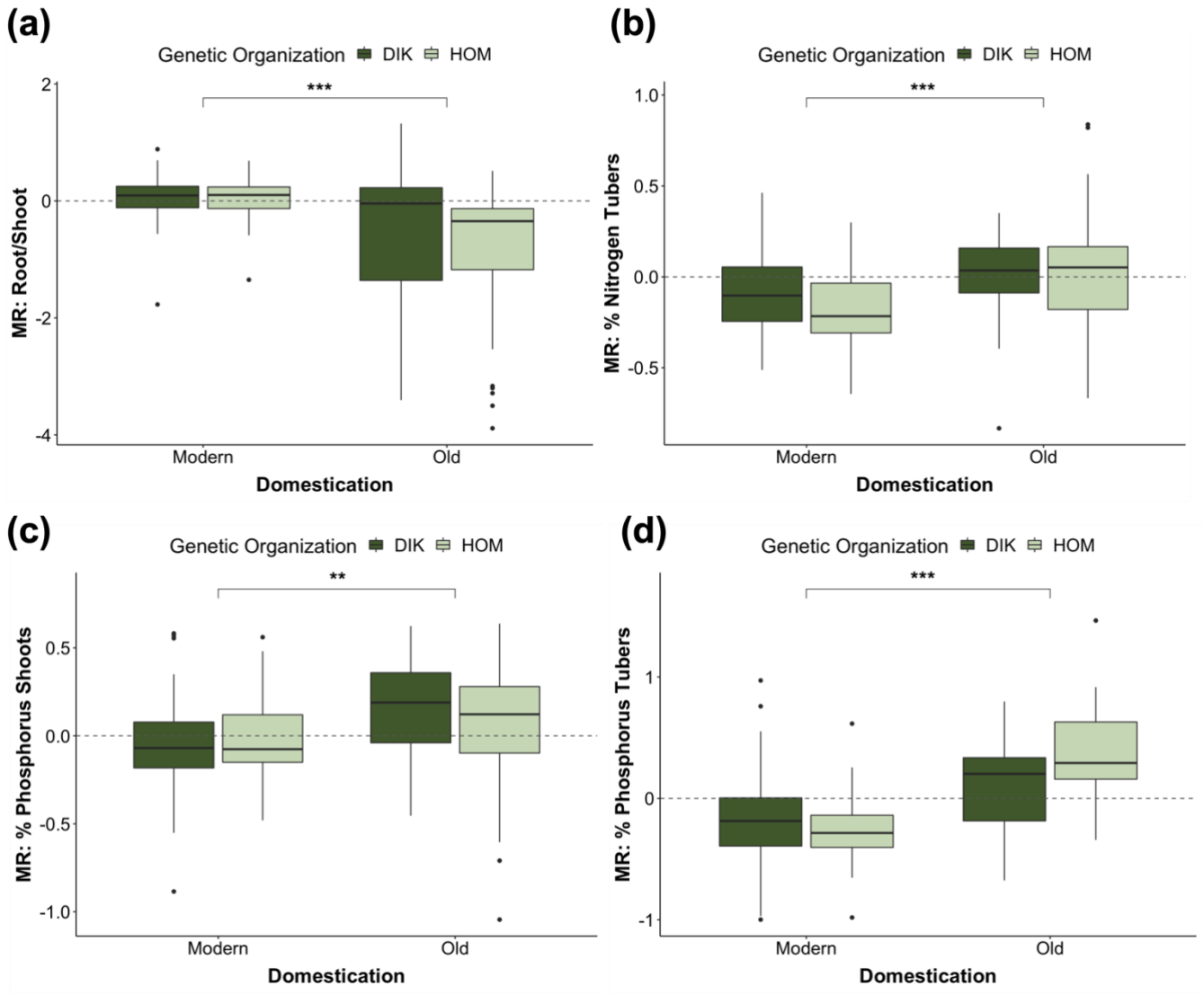


Figure 2.4. Mycorrhizal response of modern and old potato cultivars inoculated with dikaryotic (dark green) and homokaryotic (light green) strains of AMF. (a) root:shoot ratio, (b) nitrogen in tubers, (c) percent phosphorus in shoots, (d) percent phosphorus in tubers. Dotted line indicates the value of the uninoculated control plants. Values above the dotted line indicate greater than the control and values below the dotted line indicated less than the control. Mycorrhizal response (MR) is calculated as MR = ln (A/B) where A = response of individual mycorrhizal plants and B = mean response of non-mycorrhizal plants. Boxplots show the third quartile and first quartile (box edges), median (middle line), the range of the data (whiskers) and data outliers (black dots). Modern-DIK n = 43, modern-HOM n = 35, old-DIK n = 39, old-HOM n = 44. Asterisks indicate a significant difference between the MR of homokaryotic treatments and dikaryotic treatments. * signifies $p<0.05$, ** signifies $p<0.01$, ***signifies $p<0.001$.

2.5.3 Fungal responses

Colonization

With respect to % arbuscules, there is a significant interaction between domestication of cultivar and genetic organization of AMF ($p<0.001$). Specifically, when paired with modern cultivars the dikaryotic fungi have lower % arbuscules than the homokaryotic fungi, but when paired with the old cultivars these strains have higher % arbuscules than the homokaryotic strains (fig. 2.5). Genetic organization and domestication alone did not have a significant effect on % arbuscules ($p = 0.32$ and $p = 0.084$, respectively), or on % colonization ($p = 0.36$ and $p = 0.96$, respectively) and % vesicles ($p = 0.39$ and $p = 0.73$, respectively; Table S2).

Spore abundance

There was not a significant difference between the abundance of spores from homokaryotic and dikaryotic strains ($p = 0.59$). There was also not a significant difference between the abundance of spores inoculated in modern and old cultivars ($p = 0.10$).

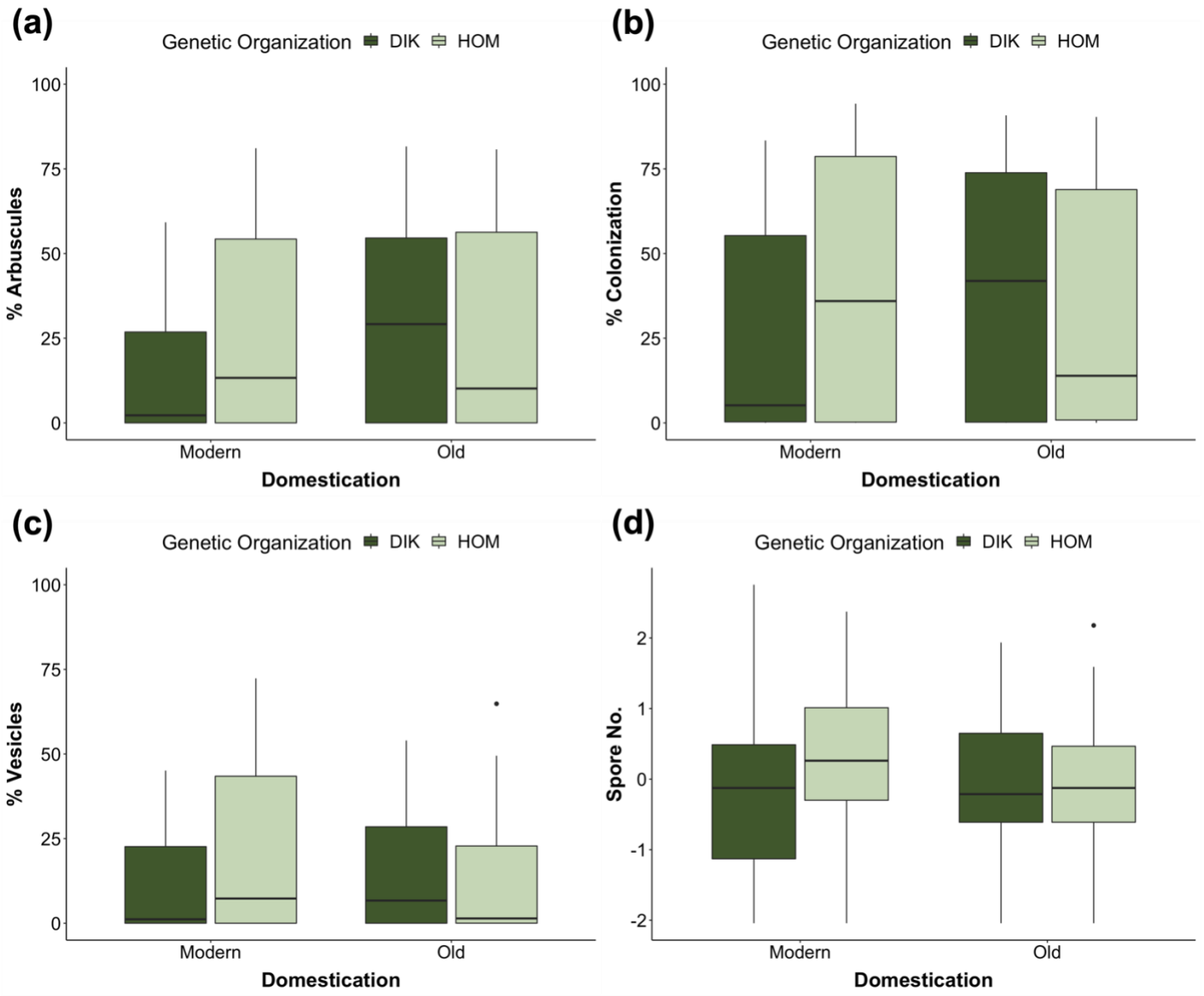


Figure 2.5. Fungal responses of dikaryotic (dark green) and homokaryotic (light green) AMF when associating with modern and old potato (*S. tuberosum*) cultivars. (a) percent arbuscules, (b) percent colonization, (c) percent vesicles, (d) number of spores. Boxplots show the third quartile and first quartile (box edges), median (middle line), the range of the data (whiskers) and data outliers (black dots). Modern-DIK n = 43, modern-HOM n = 35, old-DIK n = 39, old-HOM n = 44.

Relative Nucleotype Abundance

Plant host identity had a significant effect on the relative nucleotype abundance of dikaryotic AMF. Specifically, when strain G1 was paired with the Red Gold cultivar spores had significantly lower ratio of MAT5:MAT1 nuclei compared to spores from the other three hosts ($p < 0.001$; fig. 2.6a). Similarly, when A4 was paired with the Red Gold cultivar, spores had a significantly higher ratio of MAT1:MAT2 nuclei compared to spores from the other four hosts which were closer to an equal ratio of the two nucleotypes ($p < 0.001$; fig. 2.6b). Notably, plant host identity did not have a significant effect on the relative nucleotype abundance of strain A5 ($p = 0.11$; fig. 2.6c). Spores extracted from SL1 did not have enough material to read, therefore were excluded from the results.

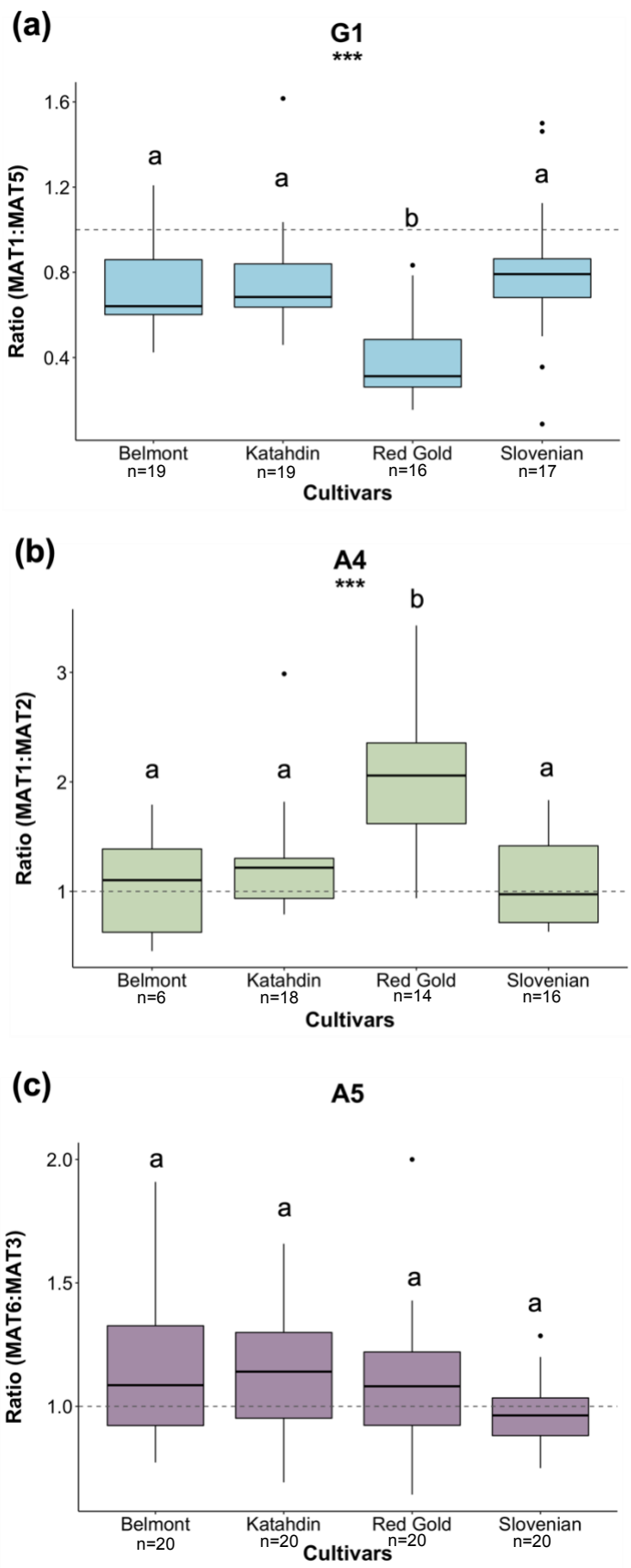


Figure 2.6. Host effect on nucleotype abundance in AMF dikaryons. The ratio of nucleotype A and B in each dikaryotic strain; (a) G1, (b) A4, and (c) A5 when paired with four potato (*Solanum tuberosum*) cultivars (AC Belmont, Katahdin, Red Gold, and Slovenian Crescent). The dotted line indicates an equal (1:1) ratio of each nucleotype. The data is based on single spore analysis. Boxplots show the third quartile and first quartile (box edges), median (middle line), and the range of the data (whiskers), and data outliers (black dots). Stars indicate statistical significance of $p < 0.001$. Letters above each boxplot indicate pairwise statistical differences based on Wilcoxon signed rank pairwise test.

2.6 Discussion

How does genetic organization impact plant productivity?

Here, we have investigated how AMF hosts respond to inoculation with dikaryotic and homokaryotic AMF strains and found that dikaryotic and homokaryotic inoculation leads to distinct mycorrhizal responses in potato plants (*S. tuberosum*). These results support the view that genome organization in AMF significantly affects MR (Kokkoris et al., 2021, 2020; Serghi et al., 2021).

Specifically, we found that homokaryotic inoculation leads to greater host biomass (total biomass, and tuber biomass) and higher number of tubers compared to dikaryotic inoculation. This contrasts with the expectation that dikaryotic strains would provide a greater advantage to the host than homokaryotic strains due to the higher genetic diversity in dikaryotic AMF. Because dikaryotic AMF have a significantly higher number of nuclei (Kokkoris et al., 2021), dikaryotic AMF may have a higher carbon demand than homokaryotic AMF leading to reduced biomass. Alternatively, the genetic identity and genome content of the homokaryotic strains we have used in this study may be particularly well suited to increase host biomass. – i.e., these strains may carry and express genes specifically needed by the plant, or proteins involved in the molecular dialogue between the partners of the mycorrhizal symbiosis.

How does domestication affect responses to distinction in genetic organization?

Plant domestication has interrupted the mycorrhizal relationship, as observed across studies (Abbo et al., 2022; Hetrick et al., 1992; Pérez-Jaramillo et al., 2015; Xing et al., 2012). Here, we found that plant domestication status also impacts how the different genetic organizations affect the mycorrhizal response. Homokaryotic AMF inoculation leads to greater distinction in mycorrhizal

response (shoot biomass and % tuber P) between old and modern cultivars, such that in old cultivars shoot biomass response and % tuber P response is high, and in modern cultivars shoot biomass response and % tuber P response is very low. In contrast, MR is significantly more stable between old and modern cultivars following inoculation with AMF dikaryons. This may be due to dikaryotic AMF being able to shift their nucleotype abundance according to host identity, allowing for rapid adaptation to change, presumably via differential gene expression as observed in other fungal phyla (Gehrmann et al., 2018; James et al., 2008). This effect may also be caused by any number of nuclei interactions such as complementation between the two nuclei allowing proteins that are unique to each nucleotype to be expressed (Ingold and Hudson, 1993).

When we look at fungal traits, we see that dikaryotic AMF have high arbuscule abundance in old cultivars and low abundance in modern cultivars, however, dikaryotic inoculation induces a similar MR (% tuber P) between old and modern cultivars, which is intriguing, given that arbuscules are the site of nutrient exchange (Smith and Read, 2008). Conversely, homokaryotic AMF had a similar abundance of arbuscules between modern and old cultivars. The exchange of nutrients is controlled by both the plant and the AMF, in a reciprocal manner (Kiers et al., 2011), and less domesticated cultivars engage in this exchange more willingly than domesticated cultivars (Garo et al., 2021; Martín-Robles et al., 2018; Nijjer et al., 2010). With our findings, this suggests that when given the opportunity, AMF dikaryons act selfishly, developing arbuscules more heavily in the ‘easy’ old cultivars than the modern cultivars, without reciprocating with the same benefits as homokaryons (e.g., increased biomass).

Notably, extra radical spore abundance did not differ between homokaryotic and dikaryotic AMF nor between old and modern cultivars. This contrasts with in vitro experiments which found that dikaryotic AMF produced significantly more spores than homokaryotic AMF (Serghi et al., 2021). This contrasting result may be due to the loss of immature (i.e., smaller) spores in the extraction process in the present study, whereas in Serghi et al. all spores could be easily viewed and quantified in the original culture (2021). Serghi et al. found that the dikaryons are slower to germinate compared to homokaryons therefore, in the present study, the dikaryotic treatments may indeed have more spores, but smaller spores that are easily lost in the extraction process. It is also possible that in the presence of potatoes, which was not used as a host in Serghi et al, AMF strains always produces similar amounts of spores regardless of their genetic identity.

Do dikaryotic AMF regulate their relative nucleotype abundance depending on host identity?

Consistent with recent work obtained using root organ cultures (ROCs) (Kokkoris et al., 2021), we found that the relative nucleotype abundance can shift significantly depending on the genetic identity of an economically important crop in a greenhouse environment. This variation in nuclear dynamics, which is particularly significant in G1 and A4, could reflect a need from the plant for specific proteins that are encoded by only one of the co-existing nuclei and need to be transcribed at higher rates – i.e., increased abundance equals higher production (through gene expression). This may lead to the more stable mycorrhizal response that we observed in the plants inoculated with dikaryotic AMF. In support of this hypothesis, unpublished data from our lab has found that the parental nuclei of these strains carry thousands of specific genes in ROCs, with each parental genotype expressing genes differently across hosts and conditions.

Does plant domestication impact mycorrhizal response?

In agreement with past research (Hetrick et al., 1992; Kokkoris et al., 2019; Zhu et al., 2001), here, we found significant overall distinction between the mycorrhizal response of modern and old cultivars. Specifically, old cultivars favoured above ground biomass, with a decreased root:shoot ratio. Plants allocate biomass where they need it most, thus favouring above ground biomass when carbon is limiting, and soil nutrients are in sufficient supply (Fitter, 1991). This suggests that the old cultivars are experiencing carbon limitations caused by AMF inoculation, whereas the modern cultivars are not. Interestingly, even though the modern cultivars have a higher root density than old cultivars, the old cultivars had increased nutrient uptake and the modern cultivars had decreased nutrient uptake. This supports the view that old cultivars are more dependent on the mycorrhiza, confirming past findings (Hetrick et al., 2011, 1992; Kokkoris et al., 2019).

2.7 Conclusion

The present study confirms that AMF dikaryons and homokaryons can distinctly affect plant hosts in greenhouse experiments. We found that homokaryotic inoculation leads to increased biomass and tuber yield, while dikaryotic inoculation leads to a more stable response (% tuber P and shoot biomass) between domestication groups. This stability may be explained by the dikaryons' ability to shift their relative nucleotype abundance between potato cultivars. Overall, old cultivars have increased nutrient uptake and greater allocation to above ground biomass compared to modern cultivars. Lastly, we found that dikaryons have a higher abundance of arbuscules in old cultivars compared to modern cultivars. These findings can be used to optimize mycorrhizal inoculum and may suggest that inoculum should be tailored to specific crops. This work can also be used to

better understand the role of genetic organization in the AMF and in its interaction with plants both in natural and in agricultural systems.

Future research should use homokaryotic strains that are genetically closely related to each dikaryotic strain as opposed to strains with similar MAT-Loci, as used here. Unpublished research has found that the homokaryotic strains used here are not closely genetically related to the dikaryotic strains (fig. S4). Genetic similarities would make comparisons between the homokaryotic and dikaryotic AMF more comparable to those of conventional dikaryons and remove confounding factors unrelated to genetic organization – i.e., evolutionary origin, genome content.

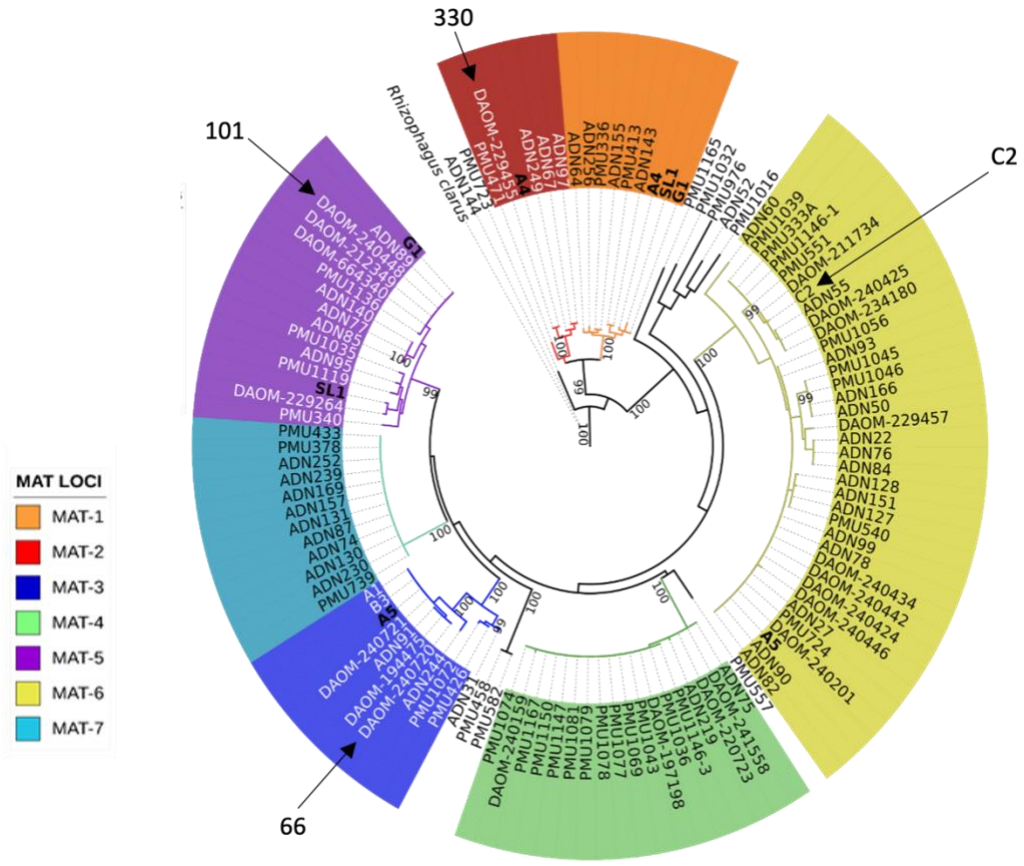
In conventional dikaryons of other fungal phyla (Ascomycota and Basidiomycota), the coexistence of divergent nucleotypes can lead to novel nuclear dynamics which lead to unique gene expression, enabling them to perform dynamically in response to their environment. Here, we found evidence that the coexistence of divergent nucleotypes in AMF dikaryons allows them to react to changes in potato host identity through shifts in relative nucleotype abundance. Dikaryotic AMF have also been found to shift their nucleotype abundance in response to abiotic stress (Cornell et al., 2022). Thus, future studies should examine the impact of genetic organization on MR under stressful conditions to determine if there would be a dikaryotic inoculation advantage for the host plants, granted by the ability of AMF dikaryons to shift nucleotype abundance in response to stress.

Importantly, future work should assess gene expression of AMF dikaryons in order to pinpoint the distinct impact of shifts in nucleotype abundance and the impact of the dikaryotic organization. This would allow us to correlate the observations made here with gene expression and better understand exactly how genetic organization is affecting the symbiosis.

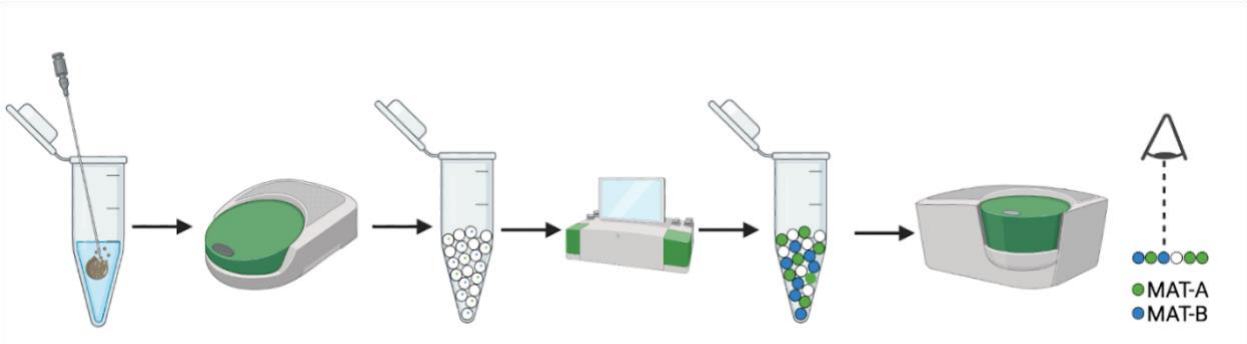
2.8 Acknowledgements

I would like to acknowledge the contributions of Kendyll Chapman, Matthew Villneuve-Laroche, Bianca Turcu, and Calvin Cornell, whose work on this project assisting with experiments and data collection was invaluable. Thank you to Vasilis Kokkoris, for your collaboration on this work. I would also like to acknowledge the work of and expertise of researchers at Agriculture and Agri-food Canada, Frank Stefani and Zhiming Zheng for work on nutrient analysis.

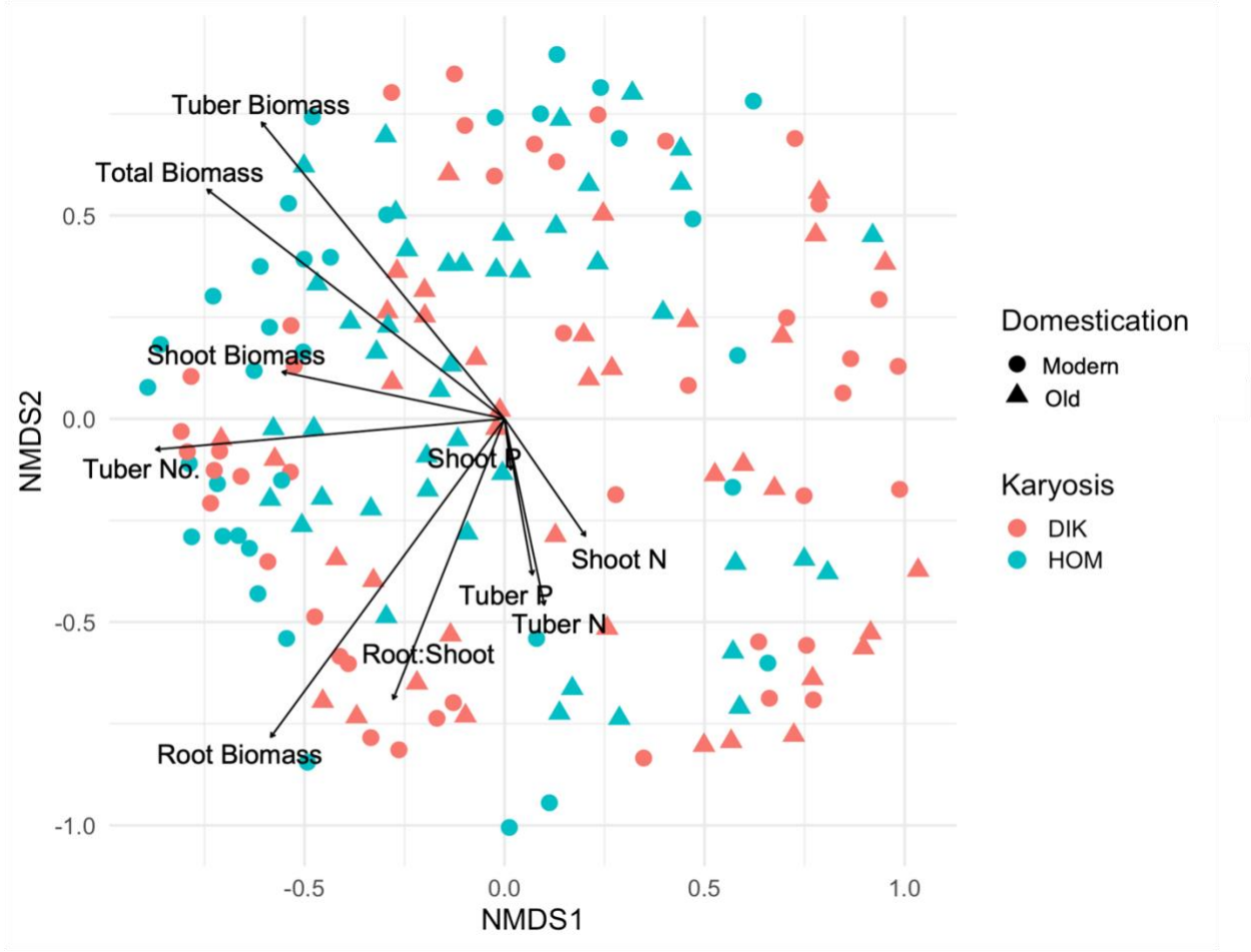
2.9 Supplementary Figures and Tables



Supplemental Figure 1. Phylogeny of 114 strains of *Rhizophagus Spp.* based on mating type (MAT) alleles. Strains are of *Rhizophagus irregularis* and the distant species *Rhizophagus clarus* MUCL-46238. Phylogeny was built based on the concatenated HD1-like and HD2 protein sequences. AMF dikaryons (A4: MAT-1,2; A5: MAT-3,6; SL1: MAT-1,5; and G1: MAT-1,5) appear in boldface. Arrows indicate homokaryotic strains chosen for the present study, selected in relation to each dikaryotic strain based on mating type. Highlighted colours represent distinct mating types. Figure modified from Kokkoris et al. (2021).



Supplemental Figure 2. Droplet digital PCR (ddPCR) procedure. Following spore isolation and washing, spores are crushed in a 0.2 mL reaction tube to release nuclei, mastermix including primer/probes are added, mixture is transferred to the droplet generator where approximately 20000 individual droplets are generated from the sample. Sample is then transferred to PCR machine where the samples are amplified within the droplets. It is then transferred to the droplet reader where individual droplets are read to detect fluorescent probes and determined to include neucleotype a (green), nucleotype b (blue), or empty (no fluorescence). Created with biorender.com



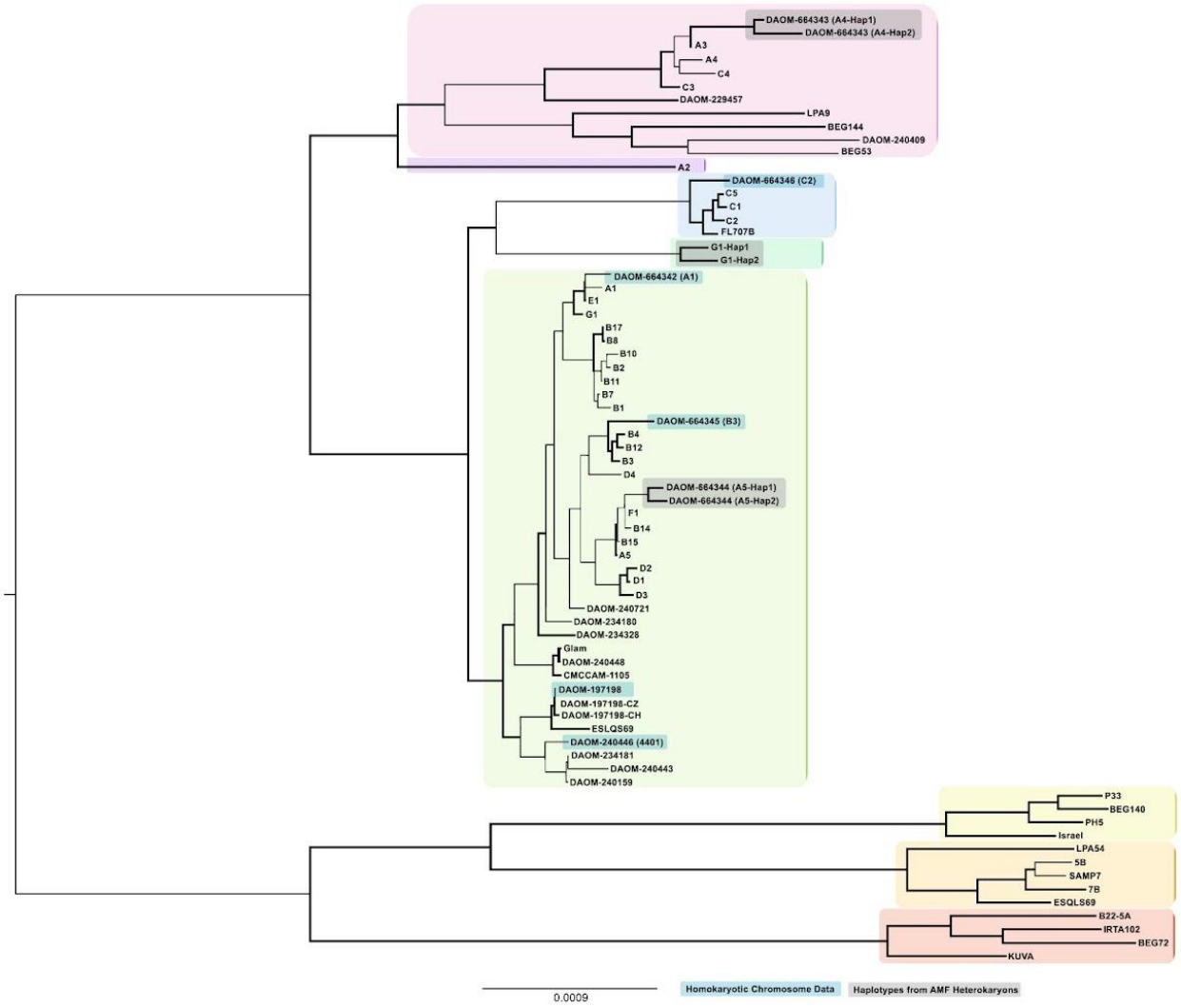
Supplemental Figure 3. Non-metric multidimensional scaling (NMDS) plot of mycorrhizal responses of homokaryotic (blue dots) and dikaryotic (red dots) AMF strains based on the measured mycorrhizal response of modern (circles) and old (triangles) potato cultivars. The direction of the traits was obtained after fitting the measured traits in the ordination space. The length of the arrow is proportional to the degree of correlation between the variable and the ordination.

Supplemental Table 1. Mycorrhizal responses of modern versus old potato cultivars inoculated with homokaryotic versus dikaryotic arbuscular mycorrhizal fungi, *R. irregularis*. Means ± standard error are presented. p-value is based on ANOVA of a linear mixed effect model of each response variable comparing the fixed factors, cultivar domestication (modern vs. old) and AMF genetic organization (dikaryotic vs. homokaryotic) and the relationship between domestication and genetic organization. Significant p - values are indicated in boldface. Mycorrhizal response was calculated Mycorrhizal response (MR) is calculated as MR = ln (A/B) where A = response of individual mycorrhizal plants and B = mean response of non-mycorrhizal plants.

							Dikaryotic		Homokaryotic		
	Dikaryotic	Homokaryotic		Modern	Old		Modern	Old	Modern	Old	
	n = 82	n = 79	p - value	n = 78	n = 83	p - value	n = 43	n = 39	n = 35	n = 44	p - value
Tuber No.	-0.098 ± 0.06	0.162 ± 0.06	**0.002**	0.049 ± 0.06	0.011 ± 0.06	0.452	-0.089 ± 0.09	-0.108 ± 0.09	0.218 ± 0.08	0.117 ± 0.08	0.879
Total biomass	0.046 ± 0.06	0.313 ± 0.05	**0.006**	0.124 ± 0.03	0.226 ± 0.07	0.268	0.066 ± 0.04	0.023 ± 0.12	0.196 ± 0.04	0.406 ± 0.08	0.081
Tuber biomass	-0.074 ± 0.06	0.170 ± 0.07	**0.003**	0.097 ± 0.05	-0.003 ± 0.07	0.489	0.029 ± 0.06	-0.188 ± 0.09	0.181 ± 0.08	0.160 ± 0.10	0.317
Root biomass	0.110 ± 0.07	0.150 ± 0.08	0.756	0.084 ± 0.05	0.173 ± 0.09	0.969	0.074 ± 0.06	0.150 ± 0.13	0.096 ± 0.07	0.192 ± 0.13	0.732
Shoot biomass	0.017 ± 0.08	0.292 ± 0.07	0.057	0.063 ± 0.03	0.236 ± 0.10	0.143	0.050 ± 0.04	-0.019 ± 0.17	0.079 ± 0.05	0.461 ± 0.10	**0.021**
Root:Shoot	-0.230 ± 0.10	-0.461 ± 0.11	0.207	0.040 ± 0.05	-0.704 ± 0.13	**< 0.001**	0.045 ± 0.06	-0.534 ± 0.19	0.034 ± 0.07	-0.855 ± 0.17	0.269
% Shoot N	0.046 ± 0.02	-0.021 ± 0.03	0.052	0.000 ± 0.02	0.026 ± 0.03	0.356	0.028 ± 0.03	0.067 ± 0.04	-0.034 ± 0.03	-0.011 ± 0.05	0.893
% Shoot P	0.053 ± 0.03	0.020 ± 0.03	0.382	-0.029 ± 0.03	0.098 ± 0.03	**0.003**	-0.035 ± 0.04	0.150 ± 0.04	-0.022 ± 0.04	0.052 ± 0.05	0.217
% Tuber N	-0.041 ± 0.03	-0.061 ± 0.04	0.445	-0.128 ± 0.03	0.022 ± 0.03	**< 0.001**	-0.088 ± 0.04	0.011 ± 0.04	-0.177 ± 0.04	0.031 ± 0.05	0.196
% Tuber P	-0.041 ± 0.05	0.087 ± 0.05	0.102	-0.206 ± 0.04	0.236 ± 0.04	**< 0.001**	-0.174 ± 0.07	0.107 ± 0.06	-0.245 ± 0.05	0.351 ± 0.06	**0.003**

Supplemental Table 2. Fungal responses of modern versus old potato cultivars inoculated with homokaryotic versus dikaryotic arbuscular mycorrhizal fungi, *R. irregularis*. Means ± standard error are presented. p-value is based on ANOVA of a linear mixed effect model (% colonization) and negative binomial generalized linear mixed effect model (% arbuscules and % vesicles) of each response variable comparing the fixed factors, cultivar domestication (old vs. modern) and AMF genetic organization (homokaryotic vs. dikaryotic) and the relationship between domestication and genetic organization. Significant p - values are indicated in boldface.

							Dikaryotic		Homokaryotic		
	Dikaryotic	Homokaryotic		Modern	Old		Modern	Old	Modern	Old	
	n = 82	n = 79	p - value	n = 78	n = 83	p - value	n = 43	n = 39	n = 35	n = 44	p - value
Spore No.	157.51 ± 44.55	179.85 ± 40.09	0.592	207.19 ± 53.16	129.39 ± 27.65	0.102	174.36 ± 81.69	140.6 6 ± 36.66	242.41 ± 67.41	117.29 ± 42.07	0.312
% Colonization	31.65 ± 3.61	36.86 ± 4.19	0.36	32.83 ± 3.83	35.66 ± 3.99	0.962	26.44 ± 4.63	37.26 ± 5.53	39.54 ± 6.05	33.97 ± 5.82	0.194
% Arbuscules	21.12 ± 2.74	27.02 ± 3.27	0.322	20.19 ± 2.68	28.10 ± 3.31	0.084	14.43 ± 2.98	28.31 ± 4.47	26.23 ± 4.36	27.88 ± 4.96	**< 0.001**
% Vesicles	12.63 ± 1.75	16.92 ± 2.42	0.389	15.83 ± 2.21	13.53 ± 1.98	0.727	11.04 ± 2.33	14.35 ± 2.63	20.86 ± 3.68	12.68 ± 3.00	0.227



Supplemental Figure 4. Phylogeny of AMF *Rhizophagus irregularis* based on recent genome assemblies. Each haplotype (nucleotype) of dikaryotic strain is highlighted in grey. Future work should focus on comparing homokaryotic and dikaryotic AMF that are closely related based on phylogenies that assess whole genome assemblies instead of exclusively mating type loci. Figure modified from work in the Corradi Lab.

CHAPTER THREE

Discussion

3.1 Summary of Findings

3.1.1 Effect of genetic organization on MR

Here, we found that inoculation with *R. irregularis* dikaryotic and homokaryotic strains leads to distinct mycorrhizal responses in potato plants (*S. tuberosum*). These results support the expectation that genome organization in AMF can result in significant differences for both partners of the mycorrhizal symbiosis (Kokkoris et al., 2021, 2020; Serghi et al., 2021).

Specifically, we found that homokaryotic inoculation leads to greater host biomass (total biomass, and tuber biomass) and higher number of tubers compared to dikaryotic inoculation. Therefore, the expectation that dikaryotic inoculation would pose a greater benefit to the host compared to homokaryotic inoculation, was not supported. The increased genetic diversity of dikaryotic AMF was predicted to provide greater benefit to the host plant, however, the higher number of nuclei in dikaryotic strains (Kokkoris et al., 2021) may instead require a higher carbon supply from the plant, leading to the reduced biomass compared to homokaryotic treatments. Alternatively, the genetic identity of the homokaryotic strains may be particularly well suited to increase host biomass. For example, the homokaryotic strains may have high expression or abundance of specific genes that are suited to increase host biomass such as those that increase the uptake of limiting-nutrients, or unique genes involved in the molecular dialogue between partners (effectors).

3.1.2 Effect of domestication on MR

In agreement with past research (Hetrick et al., 1992; Kokkoris et al., 2019; Zhu et al., 2001), here, we found significant overall distinction between the mycorrhizal response of old and modern cultivars. Specifically, old cultivars favoured above ground biomass, with a decreased

root:shoot ratio. Plants allocate biomass where they need it most, thus favouring above ground biomass when carbon is limiting, and soil nutrients are in sufficient supply (Fitter, 1991). This suggests that the old cultivars are experiencing carbon limitations caused by AMF inoculation, whereas the modern cultivars are not. Interestingly, even though the modern cultivars have a higher root density than old cultivars, the old cultivars had increased nutrient uptake (% tuber P, % shoot P, % tuber N) and the modern cultivars had decreased nutrient uptake response. This suggests that the old cultivars are more dependent on the mycorrhiza, confirming past findings (Hetrick et al., 2011, 1992; Kokkoris et al., 2019; Zhu et al., 2001).

3.1.3 Interaction between genetic organization and domestication

Here, we found that plant domestication status also impacts how the two genetic organizations affect the mycorrhizal response. Homokaryotic AMF inoculation leads to greater distinction in mycorrhizal response between old and modern cultivars. Specifically, in old cultivars shoot biomass and % tuber P is high, and in modern cultivars shoot biomass and % tuber P is very low. In comparison, we found a more stable MR to dikaryotic inoculation across plant domestication. This stability may be driven by the ability of dikaryotic AMF to shift their relative nucleotype abundance depending on host identity (confirmed here). These shifts may be reflected in the expression of each nucleotype, allowing the AMF to respond and adapt to changes in their host. Specifically, each co-existing nucleotype can express genes favoured by one particular host, allowing for higher molecular versatility, as seen in other dikaryotic fungi (Ingold and Hudson, 1993).

In terms of fungal traits, interestingly, homokaryotic strains showed a similar abundance of arbuscules between modern and old cultivars compared to dikaryotic strains, which have high

abundance in old cultivars and low abundance in modern cultivars. Because arbuscules are the site of nutrient exchange (Smith and Read, 2008), it is odd to see that even though the dikaryotic AMF have high arbuscule abundance in old cultivars and low arbuscule abundance in modern cultivars, dikaryotic inoculation leads to a similar MR between old and modern cultivars in terms of nutrient uptake (and the opposite is true for homokaryotic inoculation). The exchange of nutrients between the plant host and AMF has been found to be controlled by both partners, 'rewarding' each other for increased resources by increasing the resources that they provide (Kiers et al., 2011). This may indicate that the dikaryotic AMF are able to act selfishly when paired with the old cultivars, increasing the number of arbuscules without increasing any of the mycorrhizal responses to the level that homokaryotic inoculation does. Thus, the dikaryotic strains would acquire more carbon without providing more nutrients for the old cultivars. However, this prediction is difficult to confirm without the quantification of carbon in the AMF RNA.

Notably, extra radical spore abundance did not differ between homokaryotic and dikaryotic AMF nor between old and modern cultivars. This contrasts with in vitro experiments which found that dikaryotic AMF produced significantly more spores than homokaryotic AMF (Serghi et al., 2021). This contrasting result may be due to the loss of immature (i.e., smaller) spores in the extraction process in the present study, whereas in Serghi et al. all spores could be easily viewed and quantified in the original culture (2021). Serghi et al. found that the dikaryons are slower to germinate compared to homokaryons therefore, in the present study, the dikaryotic treatments may indeed have more spores, but smaller spores that are easily lost in the extraction process. It is also possible that in the presence of potatoes, which was not used as host in Serghi et al, AMF strains always produces similar amounts of spores regardless of their genetic identity.

3.2 Future Research

The present work revealed significant differences in the way AMF homokaryons and dikaryons behave under greenhouse experiments in the presence of potato cultivars. However, the *R. irregularis* strains I used in this study are in some cases quite different from a phylogenetic perspective, and there is some evidence that some either belong to different sub-species or a part of large "species complex" (Bruns et al., 2018; Savary et al., 2018). As such, future studies that compare MR between AMF strains should aim to use strains that are genetically closely related. Unpublished research from the Corradi Lab has found that the homokaryotic strains used here are in general, not closely genetically related to the dikaryotic strains. Genetic similarities would make the homokaryotic and dikaryotic AMF more comparable and remove confounding factors unrelated to genetic organization (genome organization, gene content, epigenetics).

To clarify how the shift in nucleotype abundance specifically impacts the performance of dikaryotic AMF, future work should investigate individual nucleotype expression (i.e., if an increase in nuclei means an increase in expression). Additionally, it has been observed that dikaryotic AMF shift their nucleotype abundance depending on abiotic stresses (Cornell et al., 2022), thus the MR of homokaryotic versus dikaryotic inoculation should also be investigated under stress to determine if dikaryotic strains would pose a greater benefit to the host under stressful conditions due to its ability to shift nucleotype abundance, as opposed to the optimized conditions here.

Lastly, future research should investigate the distribution of homokaryotic and dikaryotic AMF in nature as well as the relationship between genetic organization and plant community composition and productivity. This would build on the understanding of the role of AMF genetic

organization in terrestrial ecosystems, and further clarify the distinct strategies of homokaryons and dikaryons.

3.3 Implications and Importance of Research

Research has found that AMF have high intraspecific variation in MR. Here, we found that the distinct genetic organizations of AMF strains may contribute to this variation, as there is indeed a distinction between the mycorrhizal response of plants inoculated with homokaryotic and dikaryotic AMF. The multinucleate dikaryotic organization is a unique feature to AMF and parsing out its role in relation to its host in this obligate symbiont may provide some clarity to the investigation of AMF genetic organization.

Additionally, the current study builds on the finding that dikaryotic AMF can shift their nucleotype abundance depending on host identity, as we have demonstrated this unique molecular phenomenon in an environment that more closely resembles natural and agricultural conditions.

The distinction in MR observed here may also be useful when choosing inoculum to best suit a crop. Under optimal conditions homokaryotic AMF may be a better inoculum for increasing crop yield. However, the higher stability of MR to dikaryotic inoculation between modern and old cultivars should be considered under dynamic and potentially stressful conditions such as nutrient limitation, heat stress, and drought. Under these circumstances dikaryotic inoculation may prevail in terms of MR due to their ability to shift nucleotype abundance depending on host and environmental conditions (Cornell et al., 2022; Kokkoris et al., 2021). This underpins the need to perform similar studies to this one, in the presence of abiotic stresses.

Due to the impacts of climate change including increased global temperatures, droughts and natural disasters, the agriculture industry is experiencing major strains and these strains are expected to only increase with time (Arora, 2019; Malhi et al., 2021, 2020). One of the ultimate goals of AMF research, including the present study, is to find ways to optimize AMF inoculum for use in agriculture as the benefits of AMF include increased yield, and stress tolerance. The present work has found that one of the factors to consider in the optimization of AMF inoculum is genetic organization, and that a dikaryotic organization may be better suited to counter environmental change. Just as the formula of fertilizer is tailored to individual crops, this study supports the idea that AMF inoculum should too be tailored to individual crops depending on factors including domestication status and environmental dynamics. This strategy may offer a tool to combat some of the stresses that the agriculture industry is and will be facing.

Phosphorus fertilizer is a non-renewable resource, expected to be mined to depletion within the next hundred years (Cordell et al., 2009). As the transfer of phosphorus from the soil to the plant is one of the main roles of AMF (Smith et al., 2011) the optimization of AMF inoculum use may allow for the reduction in fertilizer input while maintaining efficient crop yields; prolonging the availability of phosphorus fertilizer or alleviating the stress of its loss.

Because inoculation with both AMF genome organizations leads to increased nutrient uptake in old cultivars, the application of AMF may be advantageous in re-establishing heritage varieties. As well, the increased resource allocation to above ground biomass in AMF inoculated old cultivars suggests that AMF induces a carbon limitation while increasing soil nutrient uptake, thus using AMF-inoculated old or wild cultivars may be more effective in carbon sequestration efforts and soil restoration in nutrient poor soil, compared to domesticated plant cultivars (Asmelash et al., 2016; Rillig, 2004; Wang et al., 2016).

3.5 Concluding Remarks

Although dikaryotic AMF induce a more stable response in distinctly domesticated hosts, this stability is not necessarily better for the hosts as homokaryotic inoculation resulted in more biomass and yield. This contrasts with the heterokaryotic advantage observed in other fungal phyla and the prediction that symbiotic partners would obtain an advantage from the dikaryotic state (Clark and Anderson, 2004; Ingold and Hudson, 1993; Strom and Bushley, 2016). Regardless, the interaction between genetic organization and domestication found here confirms that the domestication status influences how genetic organization impacts the mycorrhiza. These findings can be used to optimize mycorrhizal inoculum and may suggest that inoculum should be tailored to specific crops. This work can also be used to better understand the role of genetic organization in AMF and in its interaction with plants both in nature and in agricultural systems.

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