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RESEARCH

# Mycorrhizal response of *Solanum tuberosum* to homokaryotic versus dikaryotic arbuscular mycorrhizal fungi

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## Abstract

Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts of most land plants. In these organisms, thousands of nuclei that are either genetically similar (homokaryotic) or derived from two distinct parents (dikaryotic) co-exist in a large syncytium. Here, we investigated the impact of these two nuclear organizations on the mycorrhizal response of potatoes (*Solanum tuberosum*) by inoculating four potato cultivars with eight *Rhizophagus irregularis* strains individually (four homokaryotic and four dikaryotic). By evaluating plant and fungal fitness-related traits four months post inoculation, we found that AMF genetic organization significantly affects the mycorrhizal response of host plants. Specifically, homokaryotic strains lead to higher total, shoot, and tuber biomass and a higher number of tubers, compared to dikaryotic strains. However, fungal fitness-related traits showed no clear differences between homokaryotic and dikaryotic strains. Nucleotype content analysis of single spores confirmed that the nucleotype ratio of AMF heterokaryon spores can shift depending on host identity. Together, these findings continue to highlight significant ecological differences derived from the two distinct genetic organizations in AMF.

**Keywords** Homokaryotic · Heterokaryotic · Mycorrhizal response (MR) · Potato · *Rhizophagus irregularis* · Nucleotype ratio

## Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts that colonize the roots of approximately 78% of all vascular land plants (Tedersoo et al. 2020) including most economically important crops. During this mutualistic interaction, known as arbuscular mycorrhizal (AM) symbiosis, AMF provide nutrients (Bonfante and Genre 2010) and water (Püschel et al. 2020) to the host plants in exchange for photosynthetically derived carbon; mainly in the form of lipids (Keymer et al. 2017). In addition to increased mineral nutrient uptake, AM symbiosis provides several other important benefits to hosts, including improved tolerance to both biotic and abiotic stresses such as drought, toxic elements, and diseases (M. Chen et al. 2018a, b, c; Jung et al. 2012; Liu et al. 2018; Mitra et al. 2022).

In each spore, AMF contain hundreds to thousands of nuclei, nuclei also circulate in their coenocytic hyphae (Kokkoris et al. 2020). In the model AMF species *R. irregularis*, strains recently have been found to be either dikaryotic, containing thousands of nuclei of two divergent genotypes (nucleotypes) derived from genetically distinct parental strains (Chen et al. 2018a; Ropars et al. 2016; Sperschneider et al. 2023), or homokaryotic, in which all nuclei carry identical genomes.

In dikaryotic strains, two coexisting haplotypes differ in gene content and epigenetics, resulting in parental nucleotypes acting as distinct and variable nuclear regulatory units under different conditions and hosts (Sperschneider et al. 2023). In particular, the relative expression of each parental nucleotype correlates with their relative abundance in the

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mycelium (Sperschneider et al. 2023), which is influenced by the host plant identity (Kokkoris et al. 2021a, b) and abiotic factors in root-organ cultures (Cornell et al. 2022) and remains stable per generation regardless of spore age and locality in the network. The coexistence of two nuclear types also is functionally relevant, as these strains have distinct life history strategies compared to their homokaryotic relatives, including faster extraradical hyphal growth and the ability to produce more complex extraradical hyphal networks than homokaryotic strains (Serghi et al. 2021), a trait usually associated with elevated symbiotic mutualistic quality (Bago et al. 1997). Taken together, the different life history strategies and the malleability of their haplotype abundance may represent a strategy to optimize adaptation to change and possibly the mutualistic quality of the symbiosis.

The quality of the mycorrhizal symbiosis can be estimated by the mycorrhizal response (MR). MR can be defined as any positive or negative response by the AMF-colonized host plant of a specific trait, e.g., nutrient uptake or biomass, as a consequence of inoculation with AMF (Baon et al. 1993; Janos 2007; Sawers et al. 2008; Stahlhut et al. 2023). The factors leading to neutral or negative MR (Ceballos et al. 2019; Klironomos et al. 2000; Kokkoris et al. 2019a, b; Ryan et al. 2005) are not clearly understood, but this can be predicted partially by the plant and fungal identity (Dai et al. 2014; Ryan and Kirkegaard 2012). Several studies have investigated the effects of AMF intra- and interspecific variation on MR (Duffy and Cassells 2000; Jin et al. 2013; Klironomos 2003; Klironomos et al. 2004; Kokkoris et al. 2019a, b; Stahlhut et al. 2023), but to date, no study has systematically examined the relationships between AMF genetic organization and MR.

The aim of this study is to determine how strains with different AMF genetic organization (dikaryotic versus homokaryotic strains) affect the mycorrhizal response of potato plants (*Solanum tuberosum* L.) and to investigate further whether the dikaryotic strains are able to regulate nucleotype abundance in response to host identity under in vivo (using whole plants) conditions. Potato has been shown to be highly responsive to the AM symbiosis (Black and Tinker 1977; Davies et al. 2005; Hijri 2016; McArthur and Knowles 1993). Because of their sparse root hairs and shallow root systems, potatoes are inefficient at absorbing phosphorus (Liu et al. 2018; Yamaguchi 2002). AMF mycelium acts as an extension of the root system, compensating for the potato's shortcomings (Liu et al. 2018; Smith and Read 2008). Potatoes also are highly mycorrhizal dependant (Hijri 2016), making them an ideal host to observe the effect of AMF genetic organization on MR.

Here, we hypothesized that the changing nuclear dynamics and regulation of dikaryotic strains of *R. irregularis*, along with their rapid growth and development of complex networks, will result in a higher and more stable MR in potato cultivars inoculated with dikaryotic strains than with homokaryotic strains. To test this, we conducted a greenhouse experiment in which we inoculated four potato cultivars with either four individual dikaryotic or homokaryotic strains of *R. irregularis* and examined the MR of several growth-related factors.

## Materials and methods

### Fungal material

*Rhizophagus irregularis* strains were determined to be either dikaryotic or homokaryotic based on the identification of their mating type loci (MAT) (Ropars et al. 2016). Details on each strain are provided in Table 1. Strains were propagated using *Agrobacterium* root-inducing (Ri) T-DNA transformed root organ cultures of *Daucus carota* (cultivar P68), grown in two-compartment Petri dishes filled with M medium, as previously described by Bécard and Fortin, (1988). After approximately three months of growth, spores were extracted from the fungal side of the two-compartment Petri dishes using sodium citrate buffer to dissolve the M medium (Doner and Bécard 1991). Approximately 50 (±3) spores were placed in an agar-filled Petri dish to form inoculation pucks to prevent spore runoff from subsequently inoculated pots (Martignoni et al. 2021).

### Plant material

The four potato cultivars used in this study were selected from the Canadian Potato Genetic Resources (Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, NB, Canada), based on their variation in traits and domestication background in order to capture as much variability as possible to represent a wide range of potato plants (Table 2). Each potato cultivar was propagated in vitro from a single maternal plant by clonal propagation of shoot tissue. Specifically, 2–3 leaf-shoot cuts were planted in Plant Gene Resources Multiplication Media (PGRMM), which consisted of 4.73 g of Linsmaier and Skoog pH buffered basal salts (LSP03 – CAISSON laboratories), 30 g of sucrose (Fisher Chemicals) and 7.5 g of agar (KALYS BIOTECH) per liter, with an adjusted pH of 5.7 (±0.02). The medium was autoclaved in the liquid cycle for 25 min. Plantlets were grown individually in 47 mL flat-bottomed glass culture tubes (Sigma-Aldrich), each sealed with Breathe-EASIER™ film (Diversified Biotech) to allow uniform transmission of oxygen, carbon dioxide and water vapor. Growth conditions were set at 25/22 °C with 16/8 h of light/dark cycle until root emergence. Clonal propagation allowed us to remove unwanted genetic variation among individual plants that would occur if propagated by seed and could have confounded the effects of AMF inoculation. A total of 54

**Table 1** *Rhizophagus irregularis* strains, mating type (MAT), and collection information

| Strain | DAOM | MAT | Collecting No | Collector | Collecting date | Collection site | Subculture starting date | Field host plant | Culture host plant |
|---|---|---|---|---|---|---|---|---|---|
| A4 | 664343 | MAT-1 & MAT-2 | JansaA4 | J. Jansa | 01–1999 | Experimental field, Tänikon, Hausweid, Switzerland | 1999 | *Glycine max* | *Daucus carota* |
| A5 | 664344 | MAT-6 & MAT-3 | JansaA5 | J. Jansa | 01–1999 | Experimental field, Tänikon, Hausweid, Switzerland | 1999 | *Helianthus annuus* | *Daucus carota* |
| SL1 | 240409 | MAT-1 & MAT-5 | SL-1 | S. Lerat | 11–1999 | Montérégie, Québec, Canada | 02–07-2009 | Sugarbush forest | *Daucus carota* |
| G1 | 970895 | MAT-1 & MAT-5 | 4350 | Y. Dalpé | 23–10-1998 | St-Sixte, Outaouais, Québec, Canada | 15–04-2014 | *Echinacea purpurea* | *Daucus carota* |
| 330 | 229455 | MAT-2 | T3 | A. Chapdelaine | 10–1997 | Nursery, Terrebonne, Québec, Canada | 26–10-2017 | | *Daucus carota* |
| 66 | 240720 | MAT-3 | 4695 | Y. Dalpé, G. Mitrow | 01–08-1989 | Wasaga Provincial Park, ON, Canada | 28–06-2012 | *Ammophila sp.* | *Daucus carota* |
| 101 | 240448 | MAT-5 | GC-10 | C. Plenchette | | Tunisia | 06–11-2014 | | *Daucus carota* |
| C2 | 664346 | MAT-6 | JansaC2 | J. Jansa | 01–1999 | Experimental field, Tänikon, Hausweid, Switzerland | 1999 | *Allium porrum* | *Daucus carota* |

clonal plantlets per cultivar with five fully developed leaves were used for the experiment.

## Inoculation and growing conditions

Plastic pots (2.84 L) were filled with sterilized (autoclaved in liquid cycle for 25 min) medium consisting of 75% Appalache Valley All Purpose Sand and 25% Holiday Vermiculite by volume. Inoculum pucks were placed on top of the mix, followed by the potato plantlets. The pots were then filled with the mix to cover the AMF spores and the plant roots. Control pots were prepared with agar pucks without spores. The plants were watered daily using a drip irrigation system at a rate of 2 L per hour for one min (35 mL); after 50 days, the same regimen was applied twice a day. To promote mycorrhization, a low phosphorus environment was maintained by using a low phosphorus fertilizer (ICL Peter Excel 15–5-15 Cal-Mag special) which was diluted by adding 15 mL of fertilizer to 4 L of water, and then 50 mL of the solution (containing 29.1 mg N and 4.24 mg P) was applied to each pot every 2 weeks. Irrigation was not applied on fertilizing days to prevent water from running out of the pots. 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 included to confirm AMF

**Table 2** *Solanum tuberosum* varieties and traits

| Variety | Year established | Tuber shape | Tuber skin | Abbreviation |
|---|---|---|---|---|
| Slovenian Crescent | Heritage/unknown | Fingerling | Buff | SC |
| Katahdin | 1933 | Elliptical | Buff | K |
| Red Gold | 1970 | Round-Oval | Pinkish Red | RG |
| AC Belmont | 1967 | Round-Oval | White | AB |

colonization. Colonization was assessed one month after the start of the experiment. Plants received 16 h of light per day which was a combination of natural and artificial lighting to achieve this photoperiodicity. The greenhouse temperature was maintained between 18 and 35 ˚C. The greenhouse experiment lasted from February until June 2021.

## Mycorrhizal responses (MR)

To study the effect of AM fungal inoculation on plant MR, plant biomass and nutrient status were analyzed. The benefit or detriment that the plants received from the AM fungal inoculation was evaluated as mycorrhizal response (MR) using the following formula: $MR = \ln(A/B)$ where $A$ is the response of a mycorrhizal plant and $B$ is the mean response of all non-mycorrhizal plants of the corresponding potato variety. The MR was evaluated for the total plant biomass, root biomass, shoot biomass, root: shoot ratio, tuber biomass, tuber number, and nitrogen and phosphorus concentrations in shoots and tubers.

Four months postinoculation the shoots, roots, and tubers were separated and weighed, and tubers were counted. After harvest, the samples were dried in a 70 ˚C drying oven and weighed every 3 days until the mass was unchanged from one measurement to the next. The final mass was then measured. The root: shoot ratio was determined using dry roots excluding tubers and shoots (root mass/shoot mass).

The dry tubers and shoots were ground using a coffee grinder (Proctor Silex® Fresh Grind™) and samples were analyzed at AAFC (Ottawa Research and Development Center, Agriculture and Agri-Food Canada, ON, Canada) for percent nitrogen and phosphorus in shoots and tubers. Samples were digested using the Kjeldahl method (Nelson and Sommers 1973) and percent nitrogen and phosphorus were determined using a Flow Injection Analysis Auto-analyzer (QuikChem® 8500 by Lachat Instruments).

## Fungal responses

Successful inoculation and growth differences between dikaryotic and homokaryotic strains in vivo were assessed by determining the level of intraradical colonization and by quantifying spores in soil. Trait quantification allows us to correlate any observed differences in MR with these specific fungal traits reflecting distinct AMF life history strategies.

At harvest, fresh roots of each plant were cut into 2 cm segments, homogenized, and approximately 0.5–1 g of roots was placed in cassettes and stored in distilled water. The following day, roots were stained with black Sheaffer's ink using the ink-vinegar method (Vierheilig et al. 1998). Ten to twelve root segments were placed on three glass slides per pot (minimum of 30 root segments total per pot). The percentage of root colonization and mycorrhizal structures (vesicles and arbuscules) were assessed by light microscopy and quantified using the Trouvelot method (Trouvelot 1986), which was chosen because it provides a more detailed assessment of colonization than the main alternative colonization assessment method of McGonigle (V. Kokkoris et al. 2019a, b; McGonigle et al. 1990). Noncolonized plants (indicated as fungus strain-potato variety: one SL1-AB, one SL1-SC, two 330-K, one 330 SC, and one G1-SC) were removed from the experiment. This did not compromise statistical analyses because each treatment retained at least four replications.

Spores were collected from the soil using the spore extraction protocol of Gerdemann and Nicolson (1963). Briefly, 70 mL of substrate was collected from each pot and mixed for 5 s. The mixture was filtered through 2 stacked sieves (104 µm–63 µm) to remove particles and collect spores, which then were collected from, the bottom sieve (63 µm mesh size), transferred to Falcon tubes and centrifuged at 1200 rpm. Sucrose (45%) was added to the infranatant, and the tubes were centrifuged at 960 rpm. Spores were collected from the supernatant through a 63 µm sieve. Spores were counted manually by light microscopy.

## Nucleotype abundance

To test whether host identity had an effect on the relative abundance of the dikaryotic strains in vivo, 20 spores were randomly selected using the spore extraction method described above. Relative nucleotype abundance was assessed by droplet digital PCR (ddPCR) using the method described in Kokkoris et al. (2021a, b). Briefly, individual spores were isolated, washed in autoclaved distilled water, and pipetted into 0.2 mL PCR tubes under a stereoscope. Then 2.4 µL of autoclaved distilled water was added, and the spores were crushed with a sterilized needle under a stereoscope to release the nuclei. 21.6 µL of master mix containing 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 was added to the PCR tube. Samples then were divided into approximately 20,000 droplet compartments using a QX100™ droplet generator which due to the flow cytometry process usually results in one nucleus being in each droplet. The fragmented sample is then amplified using a C1000 Touch Thermal Cycler (Bio-Rad Technologies, Inc, Mississauga, ON, Canada) with optimized cycling conditions as described in Kokkoris et al. (2021a) and Kokkoris et al. (2021b) Following amplification, droplets that contain MAT-A fluoresce in different color than the ones containing MAT-B (due to the specific probes designed to match each MAT locus). Droplets with no nuclei remain inactive. Droplet data were analyzed using the QuantaSoft™ Analysis Pro software (1.0.596; Bio-Rad)

and scored as either nucleotype A (HEX dye probe fluorescing green), nucleotype B (FAM dye probe fluorescing blue) or no signal. Primer/probes were designed based on the mating-type loci of each nucleotype by Kokkoris et al. (2021a, b), and FAM and HEX probes were used to uniquely label each nucleotype.

## Statistical analysis

The greenhouse experiment consisted of a full factorial design with 32 treatments (four potato cultivars × eight AMF strains). Non-inoculated control pots were included for each potato cultivar. Each treatment (including the controls) had six replicates arranged in six blocks, for a total of 216 experimental units. Plants that died (i.e., eight AC Belmont plants, one Katahdin plant, six Red Gold plants and 13 Slovenian Crescent plants) during the four months of growth were removed from the experiment. Statistical differences in the mycorrhizal response between (1) potato plants colonized with dikaryotic vs homokaryotic strains, (2) the potato cultivars, and (3) their interactions were tested using permutational multivariate analysis of variance (PerMANOVA) based on Euclidean distance dissimilarities calculated with standardized data. Genetic organization of *R. irregularis* strains (dikaryotic vs. homokaryotic) and host identity (cultivar) were fixed factors, while strain identity, and block were random factors. All measured MR were used as response variables, except for shoot biomass MR because its correlation coefficient with other responses exceeded 0.8.

Statistical differences in individual traits (total biomass, tuber number, % shoot P, etc.) between dikaryotic and homokaryotic strains, and among the four cultivars were tested with a linear mixed-effects model (1), with genetic organization (dikaryon vs. homokaryon) and host identity (cultivar) as fixed factors, and inoculum strain and block as random factors.

$$Y \sim N(X\beta + o, \sigma^2 W^{-1}) \quad (1)$$

where $Y$ is the response, and $\beta$ is the vector of random effects. The distribution of $Y$ is multivariate normal where $N$ is the dimension of the response vector, $W$ is a diagonal matrix of known prior weights, $\beta$ is a p-dimensional coefficient vector, $X$ is an $n \times p$ model matrix, and $o$ is a vector of known prior offset terms. The parameters of the model are the coefficients $\beta$ and the scale parameter $\sigma$ (Bates et al. 2015). Stability was calculated as the coefficient of variance ($CV = sd/m$) where $sd$ is the standard deviation of MR and $m$ is the mean MR.

Statistical differences in the relative abundance of nucleotypes within dikaryotic spores between potato cultivars were tested with a Kruskal–Wallis test because of the non-normal distribution of the data and the variation in sample size between groups. Pairwise comparisons between cultivars following the Kruskal–Wallis analysis were performed using the pairwise Wilcoxon signed rank test.

Statistical differences in the abundance of spores produced in the soil between dikaryotic and homokaryotic AM fungal strains, and between potato cultivars, were tested with a linear mixed-effects model with genetic organization (dikaryon vs. homokaryon) and host identity (cultivar) as fixed factors, and inoculum strain as a random factor. Block was not used as a random effect because its variance was zero. Data were transformed with the *log1p* function to account for the non-normal distribution of the residuals.

Statistical differences in root colonization between dikaryotic and homokaryotic AM fungal strains, and potato cultivars were tested using a linear mixed-effects model with genetic organization (dikaryon vs. homokaryon) and host identity (cultivar) as fixed factors, and inoculum strain, and block as random factors. Colonization (%), arbuscules (%), and vesicles (%) were normalized using an ordered quantile normalization transformation to allow for a normal distribution of residuals in the model.

Data standardization and normalization, linear mixed-effects model, PerMANOVA, and data visualization (non-metric multidimensional scaling [NMDS] biplots) were performed in R studio v1.4.1717 (© 2009–2021 RStudio, PBC) using the following packages: bestNormalize v1.8.3 (Peterson 2021), ggplot2 (Wickham 2016), lme4 v1.1–28 (Bates et al. 2015), stats v4.1.0 (R Core Team 2021), and vegan v2.5–7 (Oksanen et al. 2020).

# Results

## Effects of the genetic organization of the AM fungal strains on the mycorrhizal response

To examine how homokaryotic and dikaryotic strains differentially affect the mycorrhizal response of the examined potato cultivars, we looked across all response variable using PerMANOVA. The mycorrhizal response of the examined potato plants varied significantly (PerMANOVA, $p < 0.001$; Table 3, Fig. 1) between the genetic organization of the AM fungal strains (dikaryon (DIK) vs. homokaryon (HOM)), and between cultivars (Fig. S1), when all nine plant response traits were considered. Specifically, inoculation with homokaryotic strains of *R. irregularis* resulted in 1.2-fold higher total biomass, (HOM MR mean = 0.320; DIK MR mean = 0.025; $p < 0.01$, Fig. 2A), 1.19-fold higher shoot biomass (HOM mean = 0.313; DIK mean = − 0.0001; $p < 0.05$, Fig. 2C), 1.24-fold higher tuber biomass (HOM MR mean = 0.157;

**Table 3** PerMANOVA analysis of the effect of genetic organization, cultivar, and their interaction on the mycorrhizal response of potato plants to inoculation with *R. irregularis*, based on Euclidean distance

| Factor | Df | $R^2$ | $F$ | Pr (>$F$) |
|---|---|---|---|---|
| Genetic organization | 1 | 0.02565 | 5.0725 | **0.001** |
| Cultivar | 3 | 0.17530 | 11.5544 | **0.001** |
| Interaction | 3 | 0.02528 | 1.6659 | 0.058 |
| Residuals | 153 | 0.77377 | | |
| Total | 160 | 1 | | |

Bold indicates a significant effect of the factor

*Df* degrees of freedom, $R^2$ *r*-squared, *F F* test value, *Pr(>F) p* value

DIK MR mean = − 0.112; $p < 0.01$, Fig. 2B), and 1.20-fold higher number of tubers (HOM MR mean = 0.160; DIK MR mean = − 0.102; $p < 0.01$; Fig. 2D).

Potato plants inoculated with dikaryotic strains had a 1.29-fold higher root: shoot ratio than those inoculated with homokaryotic strains (HOM MR mean = − 0.563; DIK MR mean = − 0.301; $p < 0.05$; Fig. S3b). Potato plants colonized with either type of *R. irregularis* strains had a similar root biomass ($p = 0.771$; Fig. S3a), similar concentrations of nitrogen ($p = 0.512$; Fig. S3d) and phosphorus ($p = 0.130$; Fig. S3f) in the tubers, and similar concentrations of nitrogen ($p = 0.067$; Fig. S3c) and phosphorus ($p = 0.451$; Fig. S3e) in the shoots. Absolute measurements (Fig. S5, Fig. S6) and MR (Fig. S2, Fig. S4) of all above traits for individual cultivars are presented in Supplementary Table S1 and MR of individual AMF strains in Supplementary Fig. S7 and Fig. S8. Coefficient of variation showed that dikaryotic inoculation did not lead to more stable MR compared to homokaryotic (Table S2).

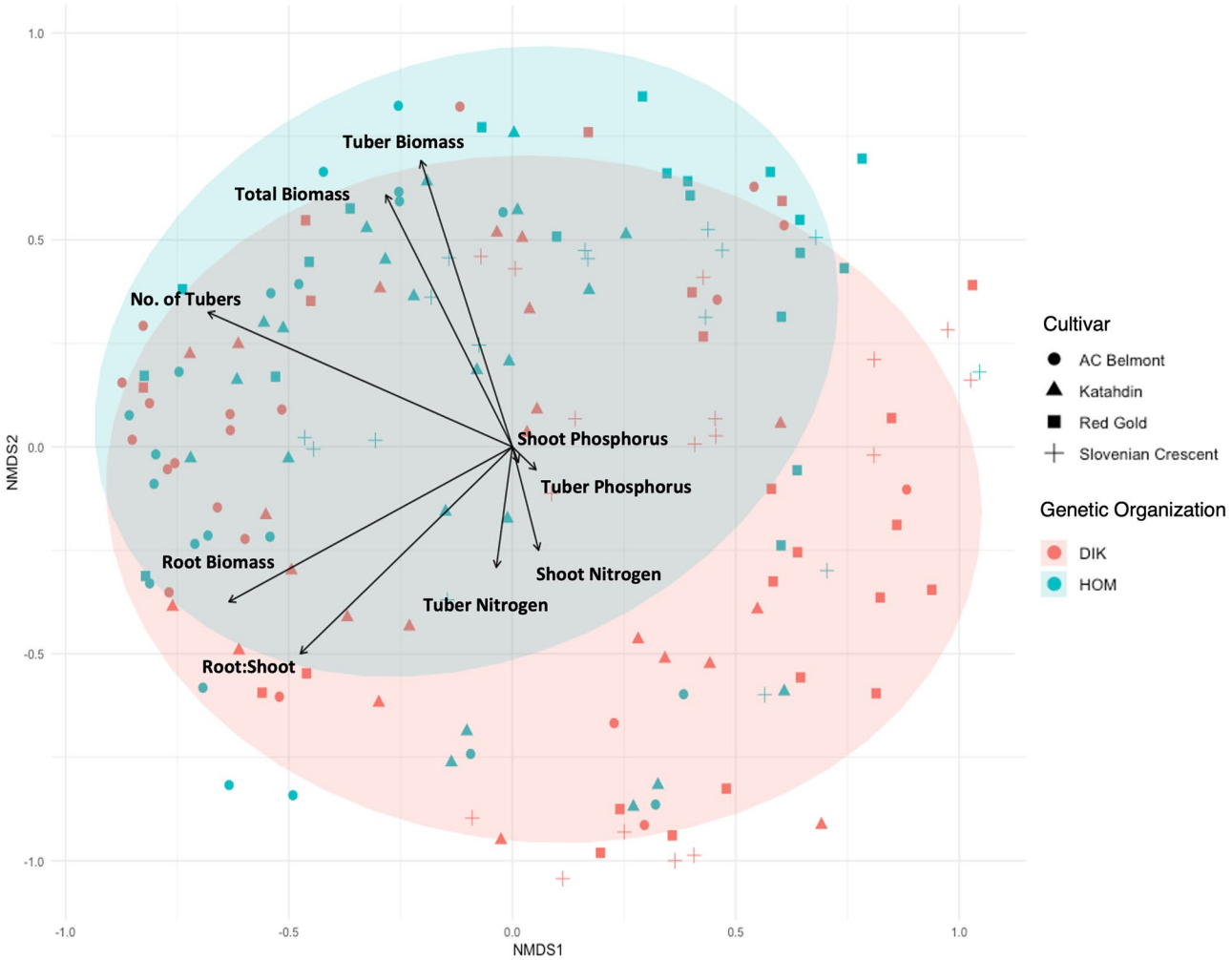


**Fig. 1** Non-metric multidimensional scaling (NMDS) plot based on Euclidean distances of the mycorrhizal responses of potato plants inoculated with homokaryotic (blue dots) or dikaryotic (red dots) strains of *R. irregularis*. Stress = 0.24. Ellipses represent 80% confidence intervals around homokaryotic (blue) and dikaryotic (red) treatments. The directions of the responses were 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. Potato cultivars are AC Belmont (circle), Katahdin (triangle), Red Gold (square), and Slovenian Crescent (+)

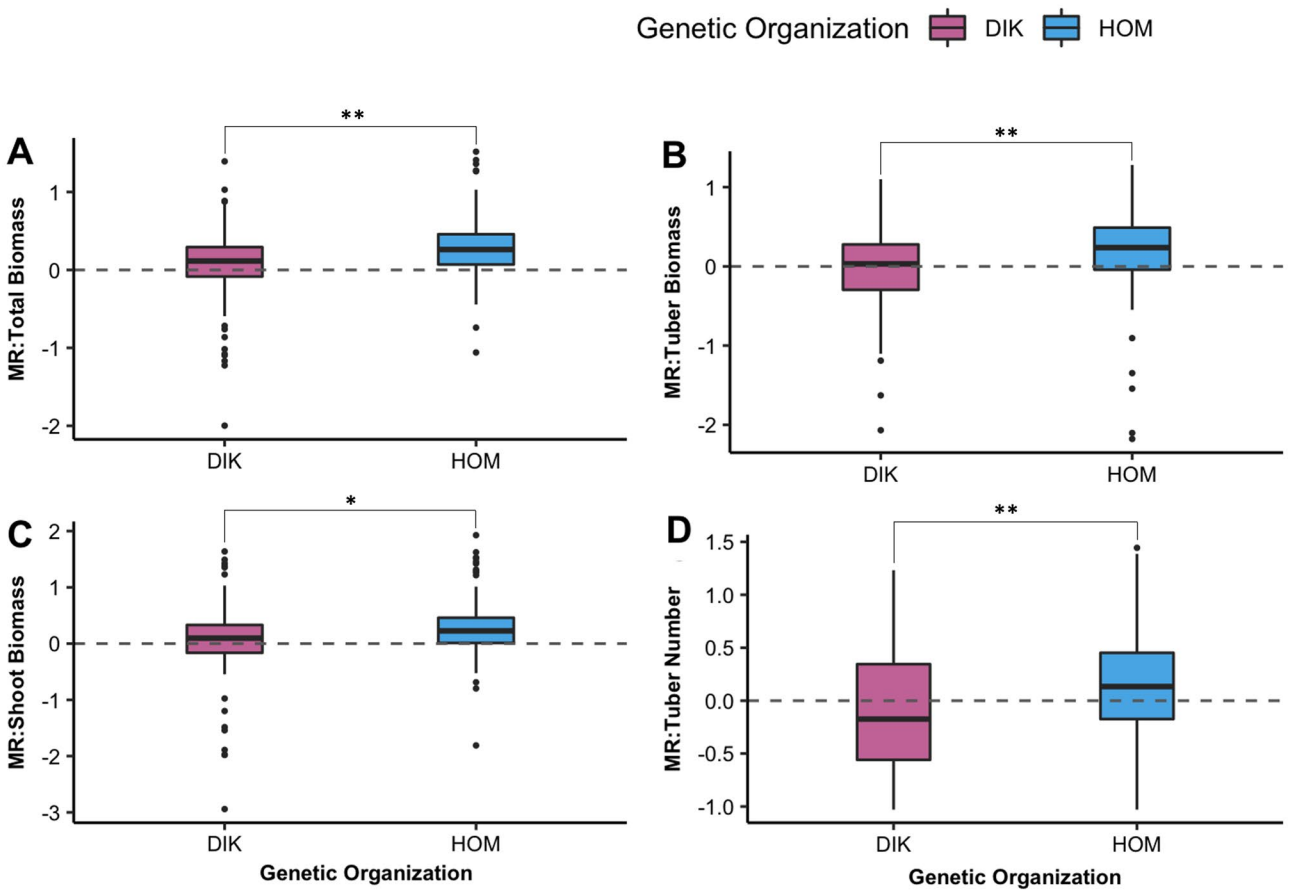


**Fig. 2** Mycorrhizal responses of four potato (*S. tuberosum*) cultivars when inoculated with dikaryotic (DIK) or homokaryotic (HOM) strains of *R. irregularis*. Boxplots show MRs of cultivars inoculated with dikaryotic (pink) or homokaryotic (light blue) strains for pooled potato cultivars. Mycorrhizal responses are total biomass (**A**), tuber biomass (**B**), shoot biomass (**C**), and number of tubers (**D**). The dotted line indicates the value of the non-inoculated control plants (i.e., no MR). Values above the dotted line indicate greater than the control, and values below the dotted line indicate 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 first and third quartiles (box edges), the median (middle line), the range of the data (whiskers), and data outliers (black dots) based on *R*'s IQR criterion. * indicates $p < 0.05$; ** indicates $p < 0.01$

## Comparative analysis of fungus life history traits between homokaryotic and dikaryotic strains

To find whether the observed differences in the MR of the examined potato cultivars could be attributed to the life history trait variation of the AMF we looked at multiple intraradical and extraradical traits. No significant differences in life history traits were observed between dikaryotic and homokaryotic strains four months after inoculation. Specifically, potato plants inoculated with either type of *R. irregularis* strains had similar levels of colonization ($p = 0.352$, Fig. 3A), and similar numbers of vesicles ($p = 0.414$, Fig. 3B), arbuscules ($p = 0.135$, Fig. 3C), and spores ($p = 0.404$, Fig. 3D). Fungal responses for individual cultivars are presented in Supplementary Fig. S9.

## Effects of potato cultivars on nucleotype abundance

To examine whether deterministic processes control the relative abundance of the nucleotypes present in the dikaryotic strains when associated with a plant in vivo, we analyzed the relative abundance of each nucleotype in individual spores using droplet digital (dd) PCR. Plant host identity had a significant effect on the relative nucleotype abundance of dikaryotic strains of *R. irregularis*. For example, the strain G1 associated with the cultivar Red Gold had a significantly lower ratio of MAT5:MAT1 nuclei across replicates ($p < 0.001$; Fig. 4A) than with the other three cultivars. Similarly, the strain A4 associated with the cultivar Red Gold had a significantly higher ratio of MAT1:MAT2 nuclei across replicates ($p < 0.001$; Fig. 4B) than with the other three cultivars, which were closer to an equal ratio of the two nucleotypes.

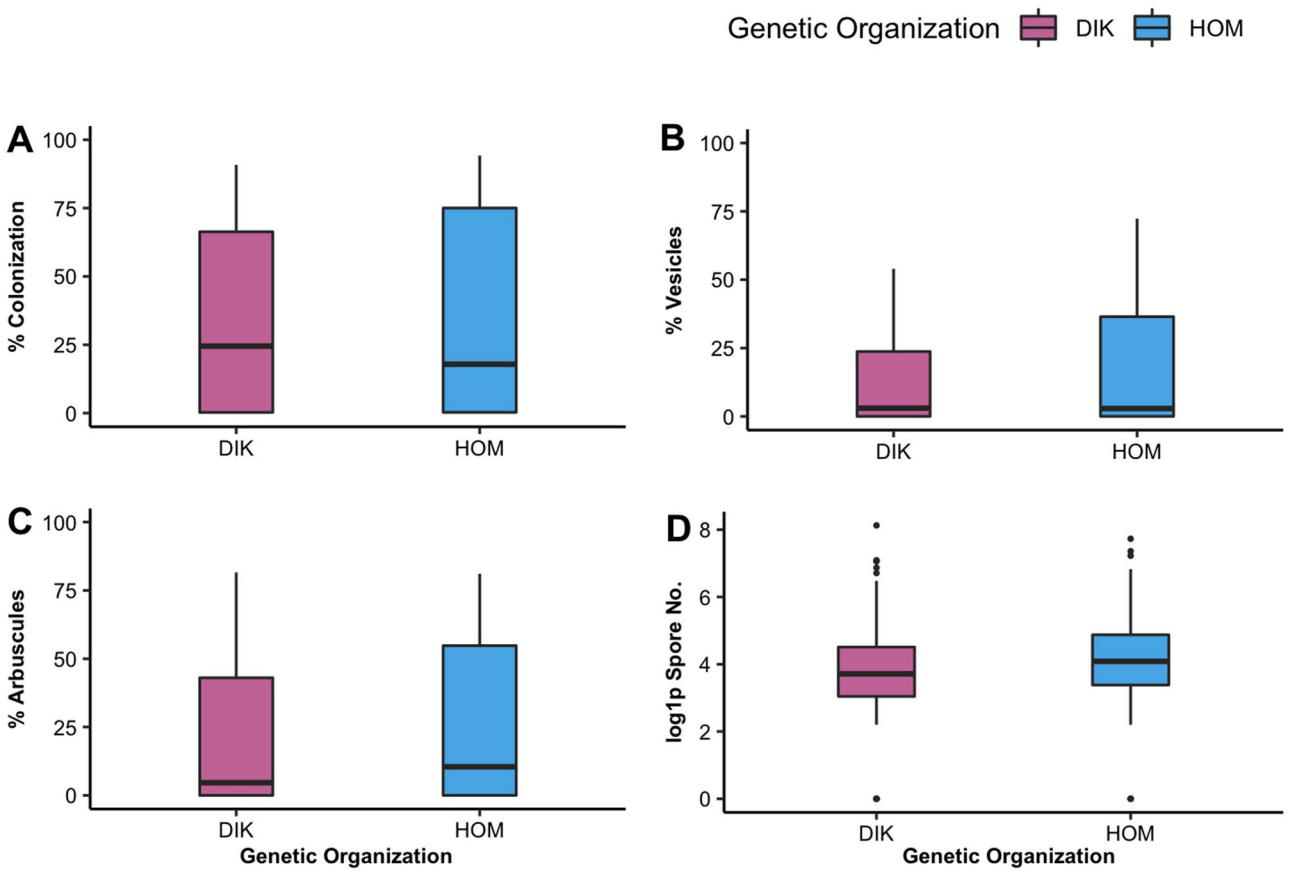


**Fig. 3** Fungal responses of dikaryotic and homokaryotic strains of *R. irregularis* when associated with four potato (*S. tuberosum*) cultivars. Boxplots show fungal responses of dikaryotic (pink) and homokaryotic (light blue) fungus strains for pooled potato cultivars. Fungal responses are percent colonization (**A**), percent vesicles (**B**), percent arbuscules (**C**), and log1p of the number of spores (natural logarithm of number of spores + 1; **D**). Boxplots show the first and third quartiles (box edges), the median (middle line), the range of the data (whiskers), and data outliers (black dots) based on *R*'s IQR criterion. None of the fungal responses differed significantly between strain types

In general, the relative abundance of nucleotypes in strain A5 was balanced across all hosts, and no significant effect on the relative nucleotype abundance was observed for this strain ($p = 0.11$; Fig. 4C). Spores extracted from SL1 appeared to be non-viable (likely devoid of nuclei) and were excluded from these analyses. What causes this condition is currently unknown but it is possible that SL1 might have a shorter lifespan that the other strains or might be less tolerant of the environmental conditions in which it was grown for this experiment.

## Discussion

### Genetic organization of R. irregularis affects host mycorrhizal responsiveness

This study provides novel evidence for the differential effects of dikaryotic versus homokaryotic strains of *R. irregularis* on the mycorrhizal responses of some potato varieties. Specifically, our results indicate that when colonized with homokaryotic strains the potato cultivars examined here (AC Belmont, Katahdin, Red Gold, and Slovenian Crescent) yield greater host biomass (total biomass, shoot biomass, and tuber biomass) and tuber production than when colonized by dikaryotic relatives. Notably, dikaryotic strains were more likely to negatively affect plant growth and did not show a more stable MR across these potato cultivars compared to homokaryotic colonization. As such, these results do not support the hypothesis that the variable nuclear dynamics and regulation of parental haplotypes in dikaryotic strains would result in more positive MR of the colonized host than homokaryotic strains.

Several possible scenarios could explain the observed differences in potato plant growth responses to dikaryotic versus homokaryotic AMF strains. First, it is possible that the higher number of nuclei in dikaryotic strains compared to

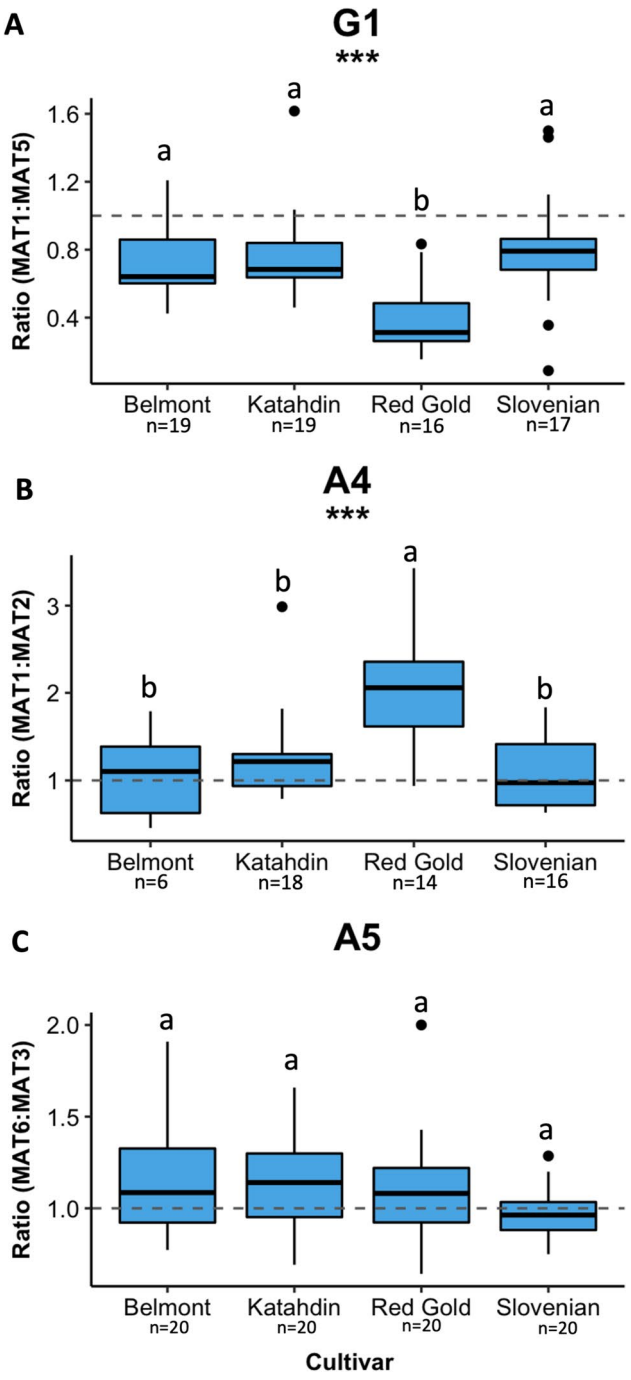


**Fig. 4** Host effect on nucleotype relative abundance in fungus dikaryons. The relative abundance of nucleotype A and B in each dikaryotic strain; (**A**) G1, (**B**) A4, and (**B**) A5 when paired with four potato (*Solanum tuberosum*) cultivars (AC Belmont, Katahdin, Red Gold, and Slovenian Crescent). The dotted line indicates an equal (1:1) ratio of each nucleotype. Data are based on single-spore ddPCR analysis. Boxplots show the first and third quartiles (box edges), the median (middle line), the range of the data (whiskers), and data outliers (black dots). *** indicates statistical significance at $p < 0.001$. Letters above each boxplot indicate pairwise statistical differences based on the Wilcoxon signed rank pairwise test

homokaryotic strains (Kokkoris et al. 2021a, b) could lead to a more "selfish" strategy in which phosphorus is retained within the fungal structures to support mitotic nuclear production rather than being made available to the host (Hammer et al. 2011; van't Padje et al. 2021b). Another possibility is that the observed difference in the fungal growth rates during the early stages of the symbiosis (Serghi et al. 2021) could initially lead to a higher carbon sink by dikaryotic strains than by homokaryotic strains, which could eventually result in reduced host biomass (or plant growth delay). Nutrient tagging techniques could help examine this hypothetical scenario in the future (Feng et al. 2020; van't Padje et al. 2021a).

The genome content of the homokaryotic strains used in this study also may be particularly well suited for increasing host biomass. Indeed, *R. irregularis* strains differ significantly in genome size, gene, and repeat content as well as in epigenetics and chromosome conformation (Chen et al. 2018b; Sperschneider et al. 2023; Yildirir et al. 2022). This extensive variability is likely related to the significant differences in phenotype and host effect observed among strains of the same species (Ehinger et al. 2009; Mathieu et al. 2018) which potentially could influence the establishment and the molecular signals between the partners of the mycorrhizal symbiosis and explain some of the observed inter-strain variability in our study.

Homokaryotic strains led to a reduced root: shoot ratio. Based on the plant driven biomass allocation for optimizing nutrient acquisition suggested by the "functional equilibrium theory," such a response means these potato plants possibly received sufficient nutrients from their homokaryotic fungal partner without having to expand their roots. Conversely, potato plants colonized with dikaryotic strains had lower shoot biomass, suggesting a higher demand for photosynthetic carbon sources by those fungus strains. It should be considered that the MR of individual plant cultivars can differ depending on phosphorus availability and cultivar vigor. As such, direct comparisons of MR between cultivars should be treated with caution.

## How does the genetic organization of AM fungal strains affect fungal responses?

Extraradical spore abundance was not significantly different between homokaryotic and dikaryotic strains of *R. irregularis* nor between cultivars. This result differs from that of Serghi et al. (2021), who observed that these dikaryotic strains produced more spores than the homokaryotic strains when grown in vitro. These also differ from what was observed for the dikaryotic strain C3 which produced significantly more spores when interacting with *D. carota* compared to the homokaryotic strains

C2 and B3 (Ehinger et al. 2009). This discrepancy may be due to differences in experimental design, as Serghi et al. (2021) and Ehinger et al. (2009) used in vitro propagation systems to visualize and quantify spore production (i.e., strains were associated with root organs in Petri dishes), whereas in the present study the strains were associated with whole plants growing in pot cultures under in vivo conditions. This may suggest that host carbon supply can strongly influence the success of the fungus (e.g., sporulation).

In addition, host identity and timing of spore analysis also may explain the differences observed in this study. For example, Serghi et al. (2021) did not use potato root organ cultures (ROCs) as a host and quantified spores 30 days after the hyphae spread into the hyphal compartment of the two-compartment Petri dishes, as opposed to four months post inoculation, so the variation in life history traits observed by Serghi et al. 2021 may be more apparent in the early growth stages of the symbiosis (Koch et al. 2017; Kokkoris and Hart 2019). Similar to our results, Ehinger et al. (2009) found no differences in spore density between the dikaryon C3 and the homokaryon C2 (also used in this study) when the strains were grown with potato roots in vitro.

### Dikaryotic strains regulate the abundance of nucleotypes depending on host identity

The ddPCR analyses we performed support the findings by Kokkoris et al. (2021a, b) obtained using ROCs, as we found that the relative abundance of nucleotypes in dikaryotic strains is not stochastic and can shift significantly depending on the genetic crop identity in a greenhouse environment. This variation in nuclear dynamics, which is particularly significant in strains G1 and A4, correlates with the relative expression and regulation of the parental haplotypes in vivo and in vitro (Sperschneider et al. 2023), and may reflect specific needs of the host plant for proteins encoded by only one of the coexisting haplotypes.

## Conclusions

The results of this study show that the nuclear types of *R. irregularis* strains (dikaryotic vs. homokaryotic) used to colonize potato plants can have a significant impact on MR. Specifically, we found that homokaryotic strains increased biomass and tuber yield in four potato cultivars.

As it has recently been shown that homokaryotic and dikaryotic strains sharing the same MAT-locus can cluster in different clades (Sperschneider et al. 2023), future studies should be aimed at focusing on comparing AMF strains with similar genetic backgrounds—i.e., clustering in similar phylogenetic clades. This approach will facilitate the assessment of mycorrhizal response in crops by minimizing confounding factors such as evolutionary origin, genome content, and relative expression. Gene expression studies should also be performed to investigate the impact of genetic organization on MR, for example, to uncover which fungal and plant genes are differentially regulated under different conditions and which are likely to be associated with increasing positive mycorrhizal responses.

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**Author contribution** N.C. and V.K. conceived and designed the experiments. V.K. maintained and propagated the potato in-vitro plantlets. V.T. maintained and propagated the AMF strains. V.T., V.K., and B.T. established the greenhouse experiment. V.T. monitored the greenhouse experiment for its duration and V.T., V.K., B.T., M.V.L., and C.C. harvested the experiment. Z.Z. performed the plant tissue nutrient analysis, K.C. quantified the spores, V.T. and M.V.L. quantified the mycorrhizal colonization and the plant biomass, V.T. performed the ddPCR analysis for nucleotype abundance. V.T. and V.K. performed the statistical analysis. V.T., N.C., and V.K. drafted the manuscript, and all co-authors discussed the results and contributed to the final version of the manuscript.

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**Availability of data and materials** Data are available as a supplemental excel file and metadata file.

## Declarations

**Ethics approval** Not applicable.

**Competing interests** The authors declare no competing interests.

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