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Arbuscular Mycorrhiza Fungi Enhance the Growth, Yield, and Quality of Medicinal Mondo Grass (*Ophiopogon japonicus* (L.f.) Ker – Gawl.) under Rainfed Conditions

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ABSTRACT

Applying the biological product of arbuscular mycorrhiza fungi (AMF) is considered an effective strategy to improve crop productivity to cope with climate change in current agricultural production. The experiment was conducted to evaluate the impact on growth, yield, and secondary metabolites of medicinal mondo grass under rainfed conditions. The split-plot design was used with the main factor of six various AMF doses (0, 100, 200, 300, 400, and 500 kg·ha^{-1} year^{-1}) and the sub-factor of two mondo grass genotypes (G1 and G2). AMF supplement had positive effects on both genotypes in increasing canopy size, the number of leaves and tillers, root growth, leaf osmotic pressure and chlorophyll fluorescence, total biomass, yield components, uptake of macronutrients, contents of polysaccharide, saponin, flavonoid, and decreasing leaf water deficit and ion leakage. G2 a higher drought-tolerant genotype performed better than G1 for investigated characteristics (except canopy height and SPAD), but the effect of AMF was clearer in the G1 genotype. Supplement of 300 kg AMF ha^{-1} year^{-1} could be the optimum rate for growth and medicinal quality of mondo grass under rainfed conditions.

Keywords: AMF, medicine, mondo grass, rainfed.

INTRODUCTION

Mondo grass (*Ophiopogon japonicus* (L.f.) Ker – Gawl.) is a valuable medicinal herb used in the pharmaceutical industry as a tonic, cosmetic, or medicinal product to treat diseases such as cough, oligogalactias, urine retention, or diabetes. Besides, it is considered an ornamental plant (Wijayabandara et al., 2015) or an intercropping plant to cover the soil and limit weeds and fungal diseases (Iqbal et al., 2004; Lin et al., 2009). In Vietnam, it has been grown as an intercropping crop with perennial orchards and industrial crops (Nguyen and Nguyen, 2011).

Climate change has been one of the challenges to agricultural production. Selecting drought-tolerant crops and improving cultivation techniques to maintain crop growth and productivity under water shortage conditions are important solutions in agricultural development strategies in the future. Mondo grass, a perennial evergreen herb, seemed to be suitable because it has a strong root system and a good drought tolerance (Zhang, 2003). Konnov and Karpun (2020) indicate the drought tolerance of *O. japonicus* through the mechanism of moisture accumulation and redistribution between vegetative organs and providing high water content in the leaves. The *OjERF* gene found in mondo grass increases drought tolerance in gen-transferred tobacco by increasing proline and active antioxidant contents (Li et al., 2012).

Arbuscular mycorrhiza fungi (AMF) are soil-dwelling fungi that enhance nutrient uptake and abiotic stress tolerance of plants (Sun et al., 2018). The infection and forming linkage of AMF with the root system increases the root surface and strengthens water and nutrient absorption from soil to increase crop yield (Bowles et al., 2016). AMF increases the drought tolerance of plants based on the development of the mycelial system to support plant uptake of water and nutrients from places where the roots cannot reach. Moreover, AMF stimulates the forming of secondary roots to maintain water balance under dry conditions (Bahadur et al., 2019). On the other hand, AMF enhances plants to increase the synthesis of antioxidant compounds, and accumulation of soluble starch, sugar, and inorganic matter to maintain water turgor pressure and metabolic functions to tolerate water deficit (Abdi et al., 2021; Bahadur et al., 2019; Hu et al., 2020). According to Newman and Reddell (1987), about 80 to 90 percent of upland plants have symbiosis relation with mycorrhizal fungi. Many previous reports admit the presence of AMF in medicinal plants such as ginger (Taber and Trappe, 1982), and turmeric (Nell et al., 2010). The effectiveness of AMF in supporting better drought tolerance was reported with many host plants such as wheat, sweet potato, pangola grass, corn, or sesame (Wahab et al., 2023). However, there is still no report on applying AMF for mondo grass. This study will support information in using AMF to support the growth, yield, and quality of mondo grass under rainfed conditions.

MATERIALS AND METHODS

Experimental design

The experiment was conducted from January 2017 to December 2018 under rainfed conditions in Bang Gia commune, Ha Hoa district, Phu Tho province, Vietnam. The experimental soil was loam sandy Plinthic Acrisols (13.3% clay, and 70.4% sand) with low pH (pH_{KCl} of 4.51), and low fertility (organic matter, total N, P_2O_5, and K_2O of 0.52%, 0.10%, 0.11%, and 0.16%). The meteorological conditions are shown in Figure 1. The average air temperature ranged from 14.5 to 31.3 °C with the highest temperature from May to September, the annual average precipitation fluctuated from 124.2 to 189.3mm with the highest rainfall from June to August, and the average air humidity ranged from 75.0 to 92.0%.

The experiment was designed in a split-plot design with three replications. Two local mondo grass genotypes (G1 and G2) with different morphological and drought-tolerant characteristics (Table 1) were assigned in the main plot. The six doses of AMF mixture including M1, M2, M3, M4, M5, and M6 with corresponding doses of 0, 100, 200, 300, 400, and 500 $kg \cdot ha^{-1}$ $year^{-1}$ were assigned in the sub-plot. The each sub-plot area was 20 m^2.

Mondo grass plants were grown on beds (30 cm in height, 2 m in width) in rows (40×20cm), respectively. The furrow drains 40 cm in width were designed according to the slope direction between the beds to drain well in heavy rain. The AMF was applied before growing (January 2017) and repeated in January 2018 with the base fertilizer of 30 kg N, 30 kg P_2O_5, and 30 kg K_2O ha^{-1} $year^{-1}$. The total amount of phosphorus (superphosphate 16%) was supplied every February. The nitrogen (urea 46%) and potassium (potassium chloride 60%) were divided to apply two times in February and July at the same amount.

Data collection

At harvest (two years after growing) 10 sample plants in each sub-plot were randomly collected to measure the growth parameters including canopy height and width; total tiller and leaf numbers; root number, length, and width. Roots and above-ground parts were dried in the oven at 80 °C until constant weight to determine dry weight. Leaf physiological parameters include osmotic pressure, water deficit, ion leakage, chlorophyll content (SPAD), and chlorophyll fluorescence (Fv/Fm). SPAD was measured by SPAD 502 – Plus meter (Minolta, Japan), and Fv/Fm was measured using Opti-Science Chlorophyll Fluorometer OS – 30p (Hudson, USA) on the 4th and 5th from the top of sample plants at 9:00 to 11:00 am. Then, ten 1 cm^2 pieces of that were prepared for each following parameter:

Leaf osmotic pressure (*P*) was calculated by the van't Hoff (1887) formula:

Table 1. Characteristics of experimental mondo grass genotypes

Genotypes	Growth and tolerant characteristics
G1	Tall plant type with dark green, large and long leaves, moderate drought tolerance and not bring *OjERF* gene
G2	Short plant type with light green, small and short leaves, drought tolerance and bring *OjERF* gene

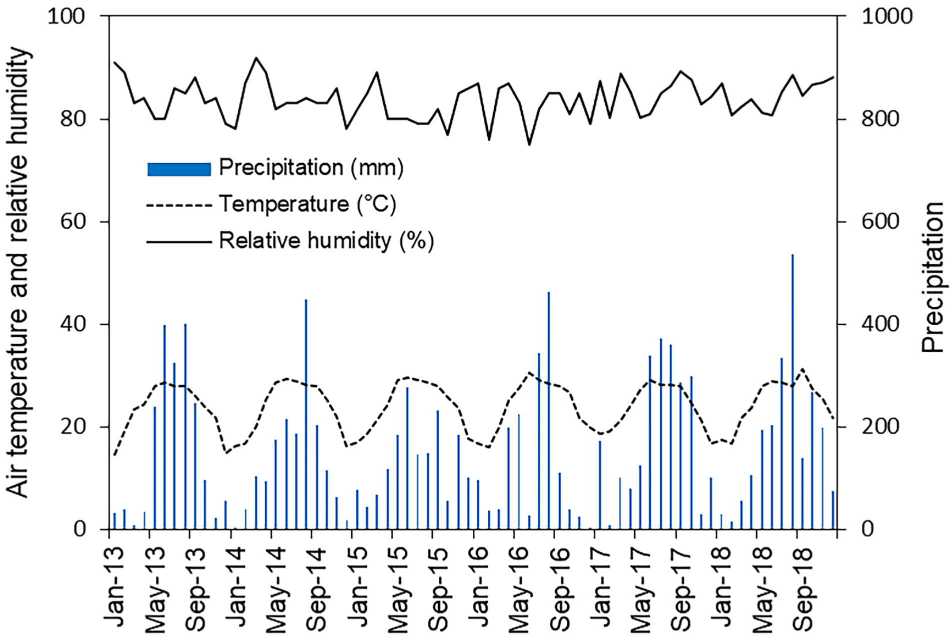

Figure 1. Five-year meteorological conditions of the experimental site 2013–2018
Source: Hydrometeorological Center of Phu Tho Province (2018)

$$P = R \times T \times C \times i \quad (1)$$

where: R is the ideal gas constant (R = 0.082), C is the molar concentration of solutes (mol L^{-1}), T is the absolute temperature (in Kelvin), and i is the isometric coefficient of the solute (i = 1 with saccharose solution).

The leaf water deficit (*LWD*) was calculated according to the formula:

$$LWD\ (\%) = [(SW - FW)/(SW - DW)] \times 100 \quad (2)$$

where: SW is the leaf-saturated weight after soaking in distilled water under dim light and controlled temperature at 24–26 °C for 24 hours, FW is the leaf fresh weight, and DW is the leaf dry weight after oven-dried at 80 °C for 48 hours or until constant dry weight.

The relative ion leakage (*RIL*) was determined using an *EC* meter AG8603 (SevenEasy, Mettler Toledo, Switzerland) according to the formula:

$$RIL(\%) = EC1/EC2 \times 100 \quad (3)$$

where: $EC1$ is the conduction of solution with leaf sample soaking in deionized water under incubation darkness condition with continuous shaking for 2 hours, $EC2$ is the conduction of solution after heated at 80 °C for 2 hours.

The tubers and roots of the sample plant were used to determine the contents of the secondary metabolites including polysaccharides, saponin, and flavonoids by the phenol-sulfuric acid method (Nielsen, 2017), the colorimetric method using a UV-VIS spectrophotometer (Benyong et al., 2014), and the $AlCl_3$ colorimetric method (Chang et al., 2002), respectively. The stover of the sample plant was ground to determine the contents of macronutrient elements by the Kjeldahl digestion method for total nitrogen, and wet oxidation method using a spectrophotometer and flame photometer for total phosphorus and potassium, respectively (Kaewpardit et al., 2009).

Data analysis

Data was collected for analysis of variance (ANOVA) according to a split-plot design using the Statistix 10.0 package. Least Significant Difference (LSD) was used to compare means.

RESULTS AND DISCUSSION

Effect of AMF on the growth characteristics of mondo grass genotypes

There were significant effects of genotypes and AMF doses on the growth of above-ground parts of mondo grass under rainfed conditions (Table 2). G1 had significantly higher canopy height but lower tiller numbers and canopy width than G2. Higher AMF doses supported better above-ground growth. The upward trends were found in the number of tillers and the number of leaves by increasing AMF doses from M1 (0 kg·ha^{-1}) to M4 (300 kg·ha^{-1}) then decreased by increasing to over 400 kg·ha^{-1} (M5). At 400 kg·ha^{-1}, applying AMF had the highest canopy height (28.8 cm), canopy width (32.4 cm), and tiller numbers (8.1 tillers hill^{-1}). The significant interaction of genotype and AMF dose showed the highest values for tiller number (8.6 tillers hill^{-1}) and leave numbers (216 leaves hill^{-1}) in the treatment combined of G2 and AMF of 400 kg·ha^{-1}, whereas the lowest values for the tiller and leaves numbers found in G1 without AMF application with 5.3 tillers hill^{-1} and 95.9 leaves hill^{-1}, respectively.

In this study, the G2 had higher root mass and total dry matter accumulation compared to the G1 under both with and without AMF supplications. The root numbers increased by raising the supplied amount of AMF from 0 kg·ha^{-1} to 300 kg·ha^{-1} before dropping at 400 and 500 kg·ha^{-1}. Similarly, there were significant increases in root length, root width, and root mass by increasing the AMF supplement. AMF had positive effects on biomass accumulation of mondo grass with differences in the response of two genotypes. Total dry matter of G1 increased from 0 (46.6 g hill^{-1}) to 500 kg AMF ha^{-1} (64.6 g hill^{-1}), but no difference was found among dry matter accumulated at 300, 400, and 500 kg·ha^{-1}. The dry matter accumulation of G2 was highest at 400 kg AMF ha^{-1} (81.1 g hill^{-1}), but the difference was not found with that at 300 kg ha^{-1}. When the amount of AMF application increased to 500 kg·ha^{-1}, dry matter accumulation was significantly lower than at 300 and 400 kg·ha^{-1}. AMF also impacted the distribution of dry matter in plants, higher AMF application had a higher ratio of dry matter between root and aboveground parts. The ratio of root and stover was highest at G2M6 (0.90) and lowest at G1M1 (0.53).

The results indicated that AMF supports the canopy and root growth of genotypes under rainfed conditions. This is in line with the report of Quiroga et al. (2020) that AMF improves maize growth with higher plant fresh weight compared to non-AMF application under both well-watered and water deficit conditions. Similarly, Rani et al., (2018) showed a significant improvement by AMF in plant height, number of productive tillers, and biomass of wheat under irrigated and drought stress conditions. Wang et al. (2018) found root length, shoot, root dry weight, and total dry weight of the medicinal *Chrysanthemum morifolium* were higher in the mycorrhizal plants than in the non-mycorrhizal plants. Plant height, plant diameter, branch length, number of branches, leave area, root length, root width, number of roots, root and total dry weight were also higher in the medicinal plant Ming Aralia supplied with AMF (Ninh and Nguyen, 2016). According to Djebaili et al. (2020) decline in indole acetic acid (IAA) generation when plant growth under water shortage conditions reduces plant growth and root elongation. Good root growth in symbiosis with AMF induces IAA generation, resulting in plant growth under water deficit conditions (Zou et al., 2017). Moreover, the carbohydrate accumulation in the root is an adaption response to develop the root system and increase the ability of water absorption (Chen et al., 2020). In mondo grass, the changes in root characteristics could be considered as adaption strategies to rainfed conditions.

Effect of AMF on the physiological characteristics of mondo grass genotypes

Without AMF application, the leaf water deficit of G1 (23.7%) was significantly higher than that of G2 (20.7%) (Table 4). This shows better leaf water maintenance of G2 compared to G1. Applying AMF significantly reduced the water deficit of both genotypes. Interaction between genotype and AMF dose showed that G2M6 had the lowest leaf water deficit (14.5%) which was followed by G1M6 (17.2%). Therefore, applying AMF enhanced root growth, and improved water uptake resulted in a lower water deficit in the plant. The result was in line with the report of Kamali et al., (2020) on sorghum that AMF improved the water use efficiency, and relative water content and decreased the electrolyte leakage and water saturation deficit in drought stress and non-stress conditions.

Similarly, AMF reduced ion leakage in both genotypes. However, the response of genotype to AMF application was different (Table 4). Ion leakage of G1 had a downward trend with increasing of AMF dose from M1 (34%) to M6 (28.8%). A significant difference was found among application doses except for ion leakage at M4 and M6. For G2, ion leakage reduced from 28.8% (M1) to M3 (26.0%) before a slight increase at M5 (26.6%) and M6 (26.2%). The difference was significant among all AMF treatments. Low ion leakage reflected low damage to the cell membrane from water deficit. This demonstrated that the AMF supplement was effective in preventing damage to cell membranes. The result is in line with the report of Begum et al., (2022) on tobacco.

The plants inoculated with AMF showed higher drought tolerance by improvement in growth and biomass throughout reducing the electrolyte leakage and lipid peroxidation and enhancing the abscisic and IAA concentration. In our study, a 300 kg AMF ha^{-1} supplement was suitable for both mondo grass genotypes to diminish the negative effects of water shortage.

Under water-shortage conditions, plants adjust the osmotic by accumulating the solutes in cells to increase cytosol osmotic pressure which stimulates more water absorption (Tran and Nguyen, 2011). Zou et al. (2021) reported that plants' symbiosis with AMF could adjust osmotic potential by changing the solute concentration to increase water uptake of the root. The experiment results showed that the osmotic pressure of G2 (3.3 atm) was significantly higher than that of G1 (2.6 atm). Increasing AMF amount increased the osmotic pressure of both mondo grass genotypes. The result aligns with reports of El-Samad et al., (2019) on maize, Begum et al., (2020) on tobacco, and Moradtalab et al. (2019) on strawberries.

The average SPAD value of G1 was 38.4 significantly higher than G2 (35.3). Applying AMF increased SPAD with the highest value at M4 on both genotypes of 41.0 and 37.0, respectively. Higher SPAD will support better photosynthetic ability. Khalill et al. (2011) reported that AMF symbiosis improves magie uptake which relates to the biosynthesis of chlorophyll. Similarly, AMF increased the chlorophyll contents in sorghum (Kamali et al., 2020) and tobacco (Begum et al., 2022) under drought stress and non-stress, SPAD in Ming Aralia (Ninh and Nguyen, 2016).

The Fv/Fm values of G2 were higher than those of G1. Fv/Fm of both genotypes increased by increasing AMF application until M4 (300 kg·ha^{-1}) but reduced at higher doses (M5 and M6). Interaction between genotype and AMF dose for Fv/Fm showed the highest value at G2M4 (0.84) and lowest at G1M1 (0.61). This suggested that the symbiosis of mondo grass with AMF could support better photosynthetic efficiency. Mo et al. (2016) demonstrated that AMF supplement enhanced watermelon drought tolerance through a better root system to ensure water absorption under water-limited conditions, stronger photosynthetic and chlorophyll fluorescence, greater protection of photosynthetic apparatus, more efficient antioxidant system, and more compatible solute accumulation to improve osmotic adjustment. Begum et al., (2002) also found increases in photosynthesis, and PSII efficiency of tobacco plants inoculated with AMF by 53.22 and 33.43% under drought stress, respectively.

Effect of AMF on the yield and quality of mondo grass genotypes

Mondo grass tubers are formed from roots, applying AMF supports better root development leading to higher tuber numbers and tuber yield. The result expressed that G1 had the lowest tuber number (52.0 tubers hill^{-1}), individual (59.0 g hill^{-1}), and actual yield (3.1 tons ha^{-1}) at the treatment of 0 kg AMF ha^{-1} (M1). The actual yield increased following the increase in AMF applications from 16.1% to 48.4%. Applying AMF of 300 kg·ha^{-1} (M4) seemed the most suitable dose with the highest actual yield of 4.6 tons ha^{-1}. However, it was significantly higher than those at 0 and 200 kg ha^{-1} (Table 2). Previous studies had the same results in using AMF to improve the yield of agricultural crops and medicinal plants under abiotic stress conditions (Begum et al., 2022; Kamali et al., 2020; Ninh and Nguyen, 2016; Quiroga et al., 2020; Wang et al., 2018). Secondary metabolite contents of G1 were significantly lower than those of G2 at all AMF treatments (Figure 2). Increasing AMF from 0 kg·ha^{-1} (M1) to 500 kg·ha^{-1} (M6) increased polysaccharide contents from 1.26 to 1.75 times, but the difference between treatment M5 and M6 was insignificant in both genotypes. Similarly, increasing AMF application increased saponin contents from 1.7 to 2.3 times in G1 and from 16.5 to 1.1 to 1.7 times in G2. However, the differences were not significant between M5 and M6 treatment in G1, and among treatment M3, M4, M5, and M6 in G2. For flavonoids, the content of this metabolite increased 1.4 to 1.6 times and reached the highest at M5 before declining at M6 in G1. Meanwhile, the flavonoid content of G2 significantly increased by increasing AMF applications for 1.3 to 2.2 times. The result agreed with Zhao et al. (2022) regarding the role of AMF in enhancing secondary compound production in plants. The same opinion was reported by Zeng et al. (2013), Ninh and Nguyen (2016), and Begum et al. (2022) that the increasing concentrations of the secondary compounds such as phenol, terpenes, alkaloids, and flavonoids in AMF symbiosis plants are a result of the stimulation of defense responses to increase tolerant ability and nutrient absorption.

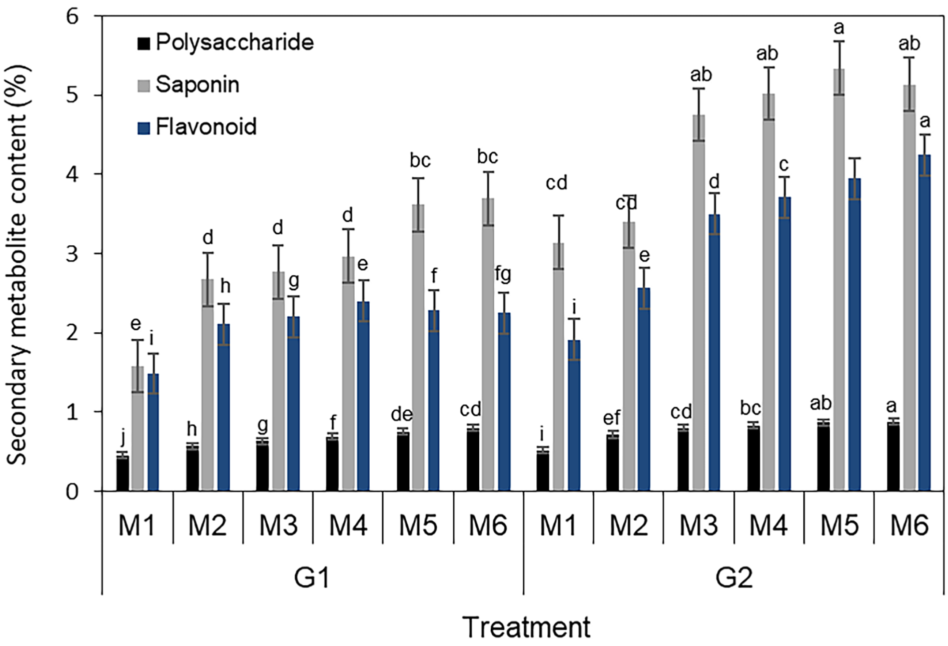

Figure 2. Effects of AMF on secondary metabolite content of mondo grass under rainfed conditions.
Note: M1, M2, M3, M4, M5, and M6 mean application dose of AMF at 0, 100, 200, 300, 400, and 500 kg AMF ha^{-1}, respectively. Different lower letter in the same column means significant differences among treatments at p≤ 0.05.

Table 2. Effects of arbuscular mycorrhizal fungi on above-ground growth of mondo grass under rainfed conditions

Treatment		Canopy height (cm)	Canopy width (cm)	Tiller number (tiller hill^{-1})	Leaf number (leave hill^{-1})
G1	M1	31.2^{e}	27.0^{i}	5.3^{h}	95.9^{k}
	M2	33.3^{c}	29.3^{f}	5.8^{g}	111.6^{j}
	M3	34.3^{b}	30.9^{c}	6.2^{f}	125.8^{h}
	M4	34.9^{a}	32.6^{a}	7.6^{d}	137.0^{g}
	M5	32.7^{d}	30.2^{d}	7.0^{e}	126.9^{h}
	M6	31.1^{e}	28.1^{h}	6.9^{e}	117.7^{i}
	Average	32.9^{A}	29.7^{B}	6.5^{B}	119.2^{B}
G2	M1	20.2^{i}	28.3^{g}	7.5^{d}	166.2^{f}
	M2	20.5^{h}	29.3^{f}	7.9^{c}	184.3^{e}
	M3	21.6^{g}	31.6^{b}	8.2^{b}	194.9^{c}
	M4	22.7^{f}	32.3^{a}	8.6^{a}	216.0^{a}
	M5	21.7^{g}	30.9^{c}	8.2^{b}	198.5^{b}
	M6	20.2^{i}	30.1^{e}	8.0^{bc}	188.7^{d}
	Average	21.1^{B}	30.4^{A}	8.1^{A}	191.4^{A}

Note: M1, M2, M3, M4, M5, and M6 mean application dose of AMF at 0, 100, 200, 300, 400, and 500 kg AMF ha^{-1}, respectively. Different lower letter in the same column means significant differences among treatments, and different capital letter in the same column means significant differences between genotypes at p≤ 0.05.

G2 uptook significantly higher N and P_2O_5 but lower K_2O. Uptake of N and P_2O_5 of both genotypes increased by increasing AMF application from M1 (0 kg·ha^{-1}) to M4 (300 kg·ha^{-1}), then declined by continuing to increase to M5 (400 kg·ha^{-1}) and M6 (500 kg·ha^{-1}). Meanwhile, K_2O contents reached the highest at the treatment of 400 kg AMF ha^{-1}, then declined at M6 in G2. It continuously increased in the G1 genotype, but no significant difference was found between M5 and M6 treatment (Figure 3). This result supported that the AMF supplement improved the

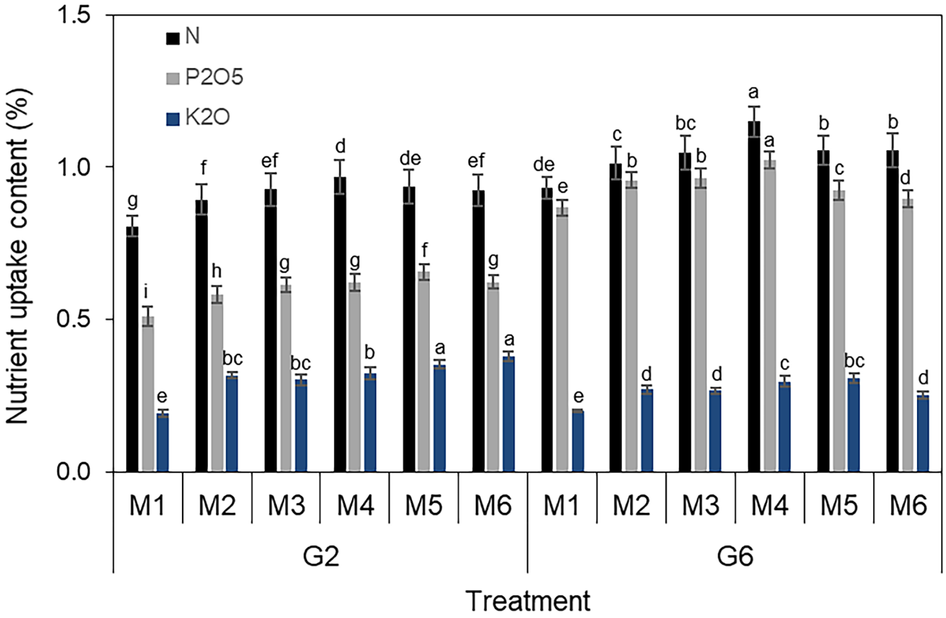

Figure 3. Effects of AMF on nutrient uptake of mondo grass under rainfed conditions
Note: M1, M2, M3, M4, M5, and M6 mean application dose of AMF at 0, 100, 200, 300, 400, and 500 kg AMF ha^{-1}, respectively. Different lower letter in the same column means significant differences among treatments at p≤ 0.05.

nutrient absorption of mondo grass. Uptake of macronutrient elements by 1.68 times for P_2O_5 and 1.2 times for N and K_2O by applying AMF. The result is consistent with the announcement of Wu et al. (2017) that AMF helps plants in better nutrient absorption under water shortage. Better N and K_2O uptake could reason for photosynthesis and water uptake under drought stress conditions (Chandrasekaran, 2020; Kamali et al., 2020; Yuan et al., 2023).

CONCLUSION

In conclusion, mondo grass genotype G2 with higher drought tolerance had better performance in growth, physiological, yield, and quality characteristics than G1 under rainfed conditions. Increasing the application rate of AMF ameliorated the growth of both genotypes with increasing plant canopy, the number of leaves, tillers and roots, root size, root mass, and total biomass. AMF showed positive effects on physiological traits with higher chlorophyll content, better leaf water status with the increase of osmotic pressure, chlorophyll fluorescence, lower leaf water deficit, and ion leakage. The tuber yield of G1 increased from 16.1% (at the rate of 100 kg·ha^{-1}) to 48.4% (at the rate of 300 kg·ha^{-1}). AMF application also enhanced secondary metabolite accumulation and nutrient uptake, especially at rates of 300 and 400 kg ha^{-1}. The results suggest the best dose of AMF supplement of 300 kg·ha^{-1} year^{-1} to improve the growth, yield, and quality of medicinal mondo grass to cope with water-limited conditions.

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