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COMBINED EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGUS INOCULATION AND SOFT ROCK AMELIORATOR ON GROWTH IMPROVEMENT OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.) UNDER DROUGHT

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Abstract

Drought stress is one of the most important factors limiting plant growth and ecosystem productivity in the Mu Us sandy land, China. A greenhouse pot experiment was conducted to investigate the effects of soft rock (SR) ameliorator, arbuscular mycorrhizal fungi (AMF) inoculation as well as their combination on growth performance of perennial ryegrass (*Lolium perenne* L.) under drought condition. The results showed that all the plant growth parameters significantly increased compared to the control plant, and the optimal plant growth performance was studied in the treatment of combination of SR ameliorator and AMF inoculation. A possible reason is that their combination directly improved water usage efficiency and indirectly improved the growth performance of perennial ryegrass. Despite using a pot experiment, the study showed significant significance on improving sandy soil and restoring pasture in the Mu Us sandy land, China.

Introduction

Environmental stress factors adversely influence plant growth and development. Among these key abiotic environmental factors, water scarcity is one of the most crucial factors limiting plant growth and ecosystem productivity (Begum *et al*. 2019). The Mu Us sandy land, one of China's four major sandy lands, is located in the northern Shaanxi Loess Plateau and the southeastern Ordos region in Inner Mongolia, with an area of 3.98×10^6 ha. Over the years, Unsustainable agricultural activities and discordant natural factors have led to soil desertification and water shortage in the area, which bring about a decline in land productivity (Sun and Han 2018). Therefore, improving the ability of soil to store water, and increasing efficiency of water utilization are indispensable strategies for sustainable development of agriculture and animal husbandry under drought conditions.

Data on various biological and chemical measures have been developed and applied to increase productivity and weaken drought effects (Cheng *et al*. 2019). Nevertheless, the effective way is to introduce SR, which is a ubiquitous mineral in Mu Us sandy land with high water retention capacity (Miščević and Vlastelica 2014). Previous research reported that SR mixed into sandy soil can significantly enhance the saturated water content and residual water content (Sun and Han 2018). Compound soil made with sand and SR supports an ascendant environment for plant growth and more than 1600 ha new cultivated lands were created by mixing SR into sandy soil in this region (Peng *et al*. 2019). Additionally, a strong relationship between plant growth and

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compounding rate has been clarified in the literature, and the treatment with rate of 2 : 1 (sandy soil: SR/ v : v) had a great effect on plant performance under drought conditions (Sun and Han 2018).

Recently, plant growth regulators arbuscular mycorrhizal fungi (AMF) have drawn much attention and involved in regulation of host plant growth under stress conditions (Ahmad *et al*. 2018). AMF are a class of soil microorganisms widely distributed in terrestrial ecosystem and can form associations with more than 80% of terrestrial plants. During past decades a renewed significance in AMF which can form rhizosphere hyphae network with the root of host plant, leading to an increased absorption and utilization of soil mineral elements and water was observed (Vander Heijden *et al*. 1998), as well as improved drought resistance (Ahmad *et al*. 2018). Considering its characteristic to stimulate growth under extreme conditions, AMF are frequently ascribed as bio-ameliorators and used to quest whether AMF improve the growth performance of host plant under stress condition (Wang *et al*. 2018, Yang *et al*. 2019).

Considering the characteristics of fast growth, multiple tillers, and excellent regenerative properties perennial ryegrass, a perennial high-quality forage grass, is often used as is an excellent variety for improving the ecological environment in arid regions (Ma 2010). Wang *et al*. (2018) showed that perennial ryegrass easily form mycorrhizae under natural conditions, and plants inoculated with AMF can adapt better to arid environments. Nevertheless, previous work was limited in exploring the effect of either AMF inoculation or SR ameliorator, and a few workers have addressed the problem on the combination use of AMF and SR. Thus present study was conducted in greenhouse conditions to investigate the potential role of AMF inoculation in combination with SR ameliorator in improving the physiological growth and lowering the water scarcity effects caused by drought in perennial ryegrass plants.

Materials and Methods

Both the matrixces soil and SR ameliorator were sampled from Dahanji village (38°27′53″N, 109°28′58″E) of Yulin city, Mu Us Sandy Land, China. The sampling site annual sunshine is 2879 hrs, and annual evaporation (280 ~ 450 mm) is greater than rainfall (1800 ~ 2500 mm), with a soil moisture content below 5% (Han *et al.* 2015). The collected matrixces soil and SR were sterilized by an autoclave (121℃ for 20 min, one-day intervals between each step) (Bahadur *et al.* 2019). The AMF (***Funneliformis coronaturm***) inoculum (spores and infected roots of *Sorghum*) was obtained from the Department of School of Life Science, Lanzhou University, Lanzhou, Gansu, P.R., China. The inoculum obtained was replicated for one year under greenhouse condition with a day-night temperature of 28/17°C. Seeds of perennial ryegrass were collected from the College of Horticulture, Northwest A and F University, Yangling, Shaanxi, P.R., China. The seeds were disinfected with 0.5% NaClO solution for 1 min, then rinsed with water, and soaked in distilled water (15 min) (Bahadur *et al*. 2019). Next the seeds were sown in Petri dish bottom which contained sponge, sprouting with a temperature of 25℃.

The experiment was arranged in a single-factor experiment with 4 levels, the treatment without any ameliorator, the treatment with 10 g AMF inoculum (approximately 2000 spores), the treatment of SR ameliorator with a mixture of SR and sand (1 : 2, v/v) and the treatment combination of SR ameliorator and AMF inoculum, hereafter referred to as CK, AMF, SR and SR/AMF, respectively. To minimize differences in microbial communities between + AMF and other treatments, 5 ml of AMF-free soil filtrate was added to AMF-free treatments (Bahadur *et al*. 2019). There were four replicates of each treatment with a total of 16 pots. Each pot (15 cm × 15 cm × 25 cm) contained 3 kg of soil substrate with equal fertility, then 10 seeds were sown per pot, respectively. After germination, the perennial ryegrass seedlings were thinned to four plants per pot. The pots were watered as needed throughout the experimental period. Modified Hoagland solution was applied to fulfill the prerequisite nutrient demand of the plants once a week. The experiment was carried out in the greenhouse of Institute of Land Engineering and Technology, Shaanxi Provincial Land Engineering Construction Group Co., Ltd., Shaanxi P.R., China from 15 May, 2019 to 30 July, 2019. The temperature was maintained between 17 and 25°C and photoperiod hours 14/10 (day/night) with 25% relative humidity and the average of 120 μ/mol/m^2 /s photosynthetic photon flux (PPF).

Plant height and tiller number were determined 0n 15, June and 30, July 1 and 30, respectively. Relative leaf chlorophyll contents were determined using a portable chlorophyll meter SPAD 502 (Minolta, Japan) (Gáborčík 2003). The second leaf from the top was selected for the net photosynthesis measurements using a portable photosynthetic system LI-COR 6400XT (LI-COR6400XT, Lincoln, NE, USA) rate from 10:00 a.m. to 12:00 p.m. on a sunny day (Ahmad *et al*. 2018). At the end of the experiment, the length, width and area of plant leaf were recorded. Afterward, shoots and roots were carefully separated. The fresh perennial ryegrass roots were cut into 1 cm segments in length and stained as described by Bahadur (2019) and mycorrhizal colonization was determined by examining root segments (50 visions were observed per segment) under the microscope. The fresh shoots were noted by weighing it on weight balance, Subsequently, the shoot was kept in the oven for 48 hrs at 80°C (Fariduddin *et al.* 2014), and then used for the determination of dry weight and shoot N and P content (Fixen *et al*. 1990). Contents of soil moisture and shoot water were determined applying gravimetric method as described by Famiglietti *et al*. (1998).

Before analysis, the raw data of those variables which were measured at four replicated pot in each treatment, were used means to represent the status of each treatment. In addition, all data were tested for normality. Statistical analyses were carried out using IBM SPSS Statistics 19.0 (StatSoft Inc., Tulsa, USA). A one-way analysis of variance (ANOVA) was used to analyze the effects of plant growth performance. The differences between the different treatments were compared using Duncan's multiple-range tests at $P < 0.05$. Dissimilarities in perennial-ryegrass growth performance among four treatments were analyzed by principal component analysis (PCA), which function from R package ‘ggbiplot’.

Results and Discussion

The colonization percentage was determined by identifying the AM typical structure including hyphae, spores, vesicle and arbuscule (Fig. 1). As compared to AMF-free plants, treatments in the presence of AMF showed high level of colonization (Table 1). Furthermore, significantly increase by 50% discovered by ANOVA ($P < 0.001$, Table 1). Results on soil moisture presented in Table 1, showed that the treatments with SR ameliorator were higher than the SR-free treatments with an increase by approximately 10%. Cheng *et al.* (2019) reported that SR ameliorator turn sandy soil to sandy loam gradually, improving the texture of the sandy soil, which favors the soil moisture content.

Results showed that all ameliorative measures significantly increased ($P < 0.001$) height and tiller number of perennial ryegrass as compared to CK at the end of the experiment (Fig. 2a, b). As shown in Fig. 2, the AMF inoculation, the SR ameliorator as well as the combination of AMF inoculation and SR ameliorator increased height by 15, 45 and 75%, respectively (Fig. 2a). The maximum tiller number of perennial ryegrass was also observed in the treatment of the combination of AMF inoculation and SR ameliorator with a highest value by 3.5 (Fig. 2b), simultaneously. Despite the AMF inoculation treatment growth was found to be slow in the early period, however, it exhibited a trend of accelerated growth from June 30 (Fig. 2a, b). Inoculation with AMF helps to establish a mutually beneficial symbiotic relationship between AMF and host plant (Vander Heijden *et al.* 1998). AM fungi provide inorganic nutrients to host plants, and host plants provide AM fungi with photosynthetic products in return (Rapparini and Peñuelas 2014), explaining why height of perennial ryegrass in AMF treatment was lower than control plant in the first 30 days after emergence.

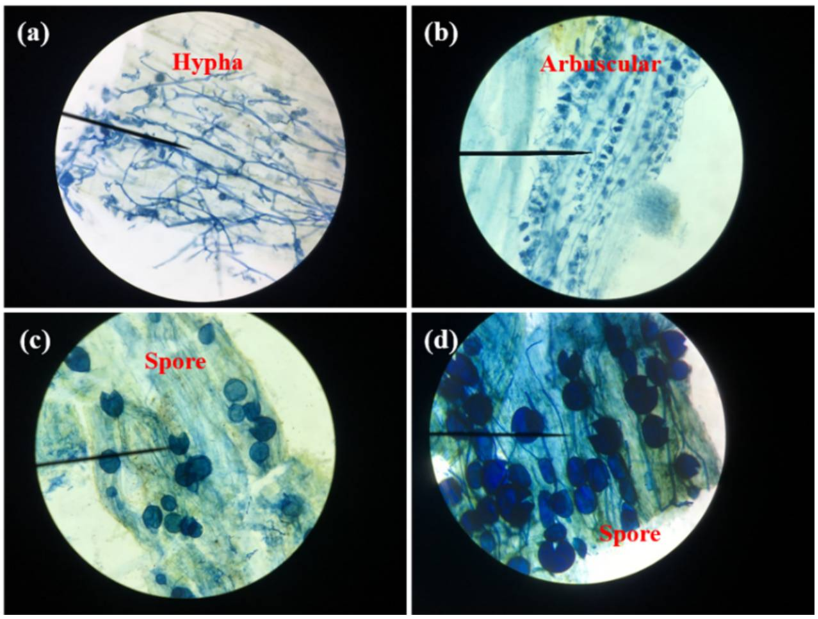

Fig. 1. Microstructure of the arbuscular mycorrhizal fungus in the root of perennial ryegrass (200×).

Table 1. AMF colonization rate as well as soil moisture content in different treatments.

Treatment	AM colonization (%)	F value	*P*	Soil moisture content (%)	F value	*P*
Ck	5.12 ± 1.11b	756.01	< 0.001***	4.12 ± 0.22b	72.31	< 0.001***
AMF	62.66 ± 2.51a			4.23 ± 0.31b		
SR	6.23 ± 1.19b			14.37 ± 0.27a		
SR/AMF	57.57 ± 3.32a			14.58 ± 3.32a		

Values in the table represent means ± SE (n = 4). Different letters indicate significant differences ($P < 0.05$).

All the improved treatments significantly increased ($P < 0.001$) shoot biomass, leaf water content, shoot N and P content of perennial ryegrass as compared to the control plants (Fig. 3). Plants which were exposed to SR ameliorator, AMF inoculation, and their combination exhibited increase of 130, 110 and 190%, respectively in shoot dry weight of perennial ryegrass compared to the control plants (Fig. 3a). Similarly, leaf water content increased significantly due to above improved treatment, with an approximate rise of 100, 100 and 150%, respectively (Fig. 3b). In addition, shoot N and P content showing the same trend, scilicet, the SR ameliorator was like AMF inoculation in terms of above plant growth parameter, which is better than the control plant, however, is inferior to their combination (SR/AMF) (Fig. 3c, d). The optimal plant growth performance was observed in the combination of AMF inoculation and SR ameliorator due to improved water holding capacity as well as the efficiency of increased water utilization. Cheng reported that SR ameliorator cause a rise of soil organic carbon content (Cheng *et al*. 2019), this is beneficial to the growth and extension of AMF hyphae (Rillig *et al*. 2001). The water and inorganic nutrients including available nitrogen (AN) and phosphorus (AP) which are indispensable for plant growth can be conveniently absorbed via AMF extra-mycelium (Wahid *et al.* 2016). Furthermore, the AMF extra-mycelium abundantly distributed in soil not only improves the soil enzyme activity in the drought stress, but also improves the soil rhizosphere

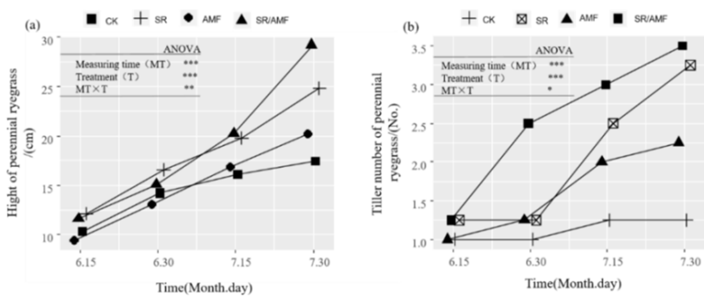

Fig. 2. Effect of SR ameliorator, AMF inoculation, and their combination on height (a) and tiller number (b) of perennial ryegrass after seeding emergence for 30, 45, 60 and 75 days, respectively. Data are means ± se (n = 4). Different letters indicate significant differences (P < 0.05).

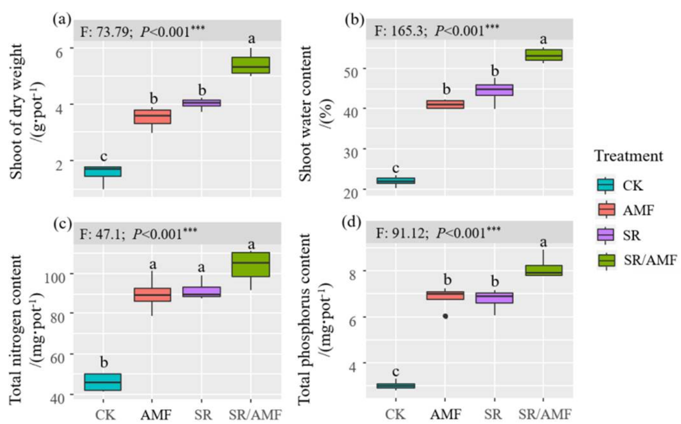

Fig. 3. Effect of SR ameliorator, AMF inoculation, and their combination on shoot dry weight (a), shoot water content (b), TN content (c) and TP content (d) of perennial ryegrass, respectively. Data are means ± se (n = 4). Different letters indicate significant differences (P < 0.05).

micro-environment, which helped in the absorption for soil available nutrients. This study showed that both N and P content significantly are affected by combination of AMF inoculation and SR ameliorator. Similar to the present finding, the AMF-induced rise of N and P uptake was reported in perennial ryegrass (Yang *et al*. 2019). Farzaneh *et al.* (2011) also reported significant rise in uptake soil available nutrients due to AMF inoculation.

All improved treatments significantly increased (P < 0.001) plant growth performance including leaf width, leaf length, leaf area, SPAD value and net-photosynthetic rate as compared to the control plant (Table 2). The optative plant growth performance was observed in the treatment of combination of SR ameliorator and AMF inoculation (SR/AMF), which increased leaf width by 150%, leaf length by 75%, leaf area by 550% and SPAD value by 150% and net-photosynthetic rate by 170%, respectively. In terms of plant growth performance, the SR ameliorator was better than AMF inoculation (AMF), however, both were inferior to their combination (SR/AMF). The results showed that the combination of AMF inoculation and SR ameliorator significantly increased relative chlorophyll content (SPAD). It is understood that inorganic elements such as N and P are important elements which participate metabolism and synthesize tissues of organs. Increased N uptake through AMF extra-mycelium may have contributed to increase synthesis of prerequisite of chlorophyll (Pettersson and McDonald 1994). In addition, increased P uptake is beneficial to ATP synthesis and metabolism of lipids. In the present study, net-photosynthetic rate was found to be significantly affected by combination of AMF inoculation and SR ameliorator. This finding is in agreement with the finding of Begum *et al*. (2019). AMF inoculation regulates the stomatal parameters and prevents photoinhibition under drought stress (Begum *et al.* 2019), which contribute to an efficient photosynthesis. Moreover, AMF-mediated increase in photo-synthetic efficiency also might be due to increased rubisco synthesis (Fatma *et al*. 2014).

Table 2. Effect of SR ameliorator, AMF inoculation and their combination on plant leaf parameter, SPAD value and net photosynthesis rate.

Treatment	Leaf width (mm)	Leaf length (cm)	Leaf area (cm^2)	SPAD	Net-photosynthetic rate ($\mu mol/m^2/s$)
Ck	2.2 ± 0.08c	12.25 ± 0.98d	2.02 ± 0.56d	21.95 ± 0.23d	1.05 ± 0.13d
AMF	2.65 ± 0.06b	15.27 ± 1.04c	5.22 ± 2.27c	39.22 ± 0.67c	1.52 ± 0.13c
SR	4.78 ± 0.09b	18.01 ± 0.97b	8.09 ± 1.01b	47.01 ± 0.11b	1.77 ± 0.09b
SR/AMF	5.3 ± 0.08a	21.32 ± 1.11a	13.41 ± 2.06a	55.22 ± 0.44a	2.72 ± 0.17a
F value	126.8	56.47	94.11	41.76	110.9
P	$< 0.001^{***}$	$< 0.001^{***}$	$< 0.001^{***}$	$< 0.001^{***}$	$< 0.001^{***}$

Data are means ± SE (n = 4). Different letters indicate significant differences ($P < 0.05$).

Principal component analysis (PCA) was performed with standardized data for plant growth performance factors. When extracting the principal components, the rules that the Eigen value is greater than 1 and the cumulative contribution rate is higher than 80% is generally followed. Table 3 shows that the value of Eigen values of first axes is 9.85, with a variance contribution rates of 89.62%, which can basically reflect the differences on plant growth properties in different treatments. The first two axes with the highest principal component scores were extracted for the subsequent mapping. As can be seen from Fig. 4, different treatments can be well separated on PC1 axis. In addition, single plant growth factors (X1-X11) contribution rates are less than mean contribution rates of all plant growth parameter (Fig. 4), indicating that all these improved treatments have more average effect on various parameters of plant growth performance.

SR ameliorator and AMF inoculation have an additive effect on perennial ryegrass under drought stress. Current findings of the present research on the SR ameliorator in combination with AMF inoculation resulted in improved plant attributes including height, tiller number, leaf width, leaf length, leaf

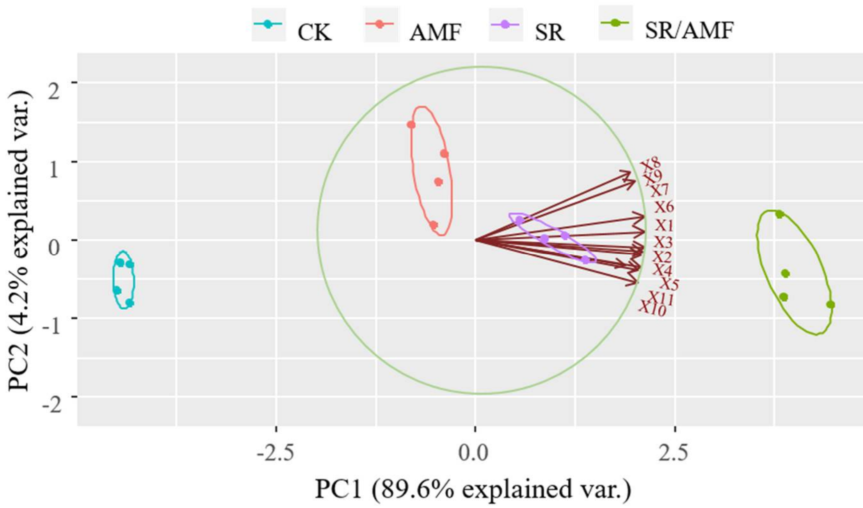

Fig. 4. PCA sequencing of different treatments with plant growth performance factors. X1, X2 … X11, represent leaf length, leaf width, leaf area, SPAD, net-photosynthetic rate, shoot of dry weight, shoot water content, TN content, TP content, height, tiller number, respectively.

Table 3. The loadings and explained variance of plant growth parameters in the first three axes in principal component analysis (PCA).

Grow performance	PCA1	PCA2	PCA3
Leaf length/cm	1.06	–0.04	0.13
Leaf width/mm	1.04	–0.91	0.01
Leaf area/cm^2	1.05	–0.07	0.10
SPAD value	1.03	–0.17	0.01
Net-photosynthetic rate/umol/m^2/s	1.02	–0.18	0.23
Shoot of dry weight/g/pot	1.05	0.05	0.03
Shoot water content/%	1.05	0.15	–.06
Total N content/mg/pot	0.97	0.43	-0.33
Total P content/mg/pot	0.99	0.38	0.01
Height/cm	1.01	–0.27	0.01
Tiller number/No.	0.94	_0.15	–0.48
Eigen value	9.85	0.45	0.28
Proportion explained	0.89	0.04	0.02
Cumulative proportion	0.89	0.93	0.96

area, shoot dry weight, SPAD value and photosynthesis as well as efficient N and P absorption. The overall finding of the experiment are illustrated graphically in Fig. 5. Despite this study was carried out through a pot experiment, which is different from natural environment, the study has an considerable theoretical guidance significance on improving sandy soil and recovering the vegetation in the Mu Us sandy land as well as semi-arid region. On a wider level, further work needs to be carried out to establish whether different AMF species have a distinct effect on resisting drought stress.

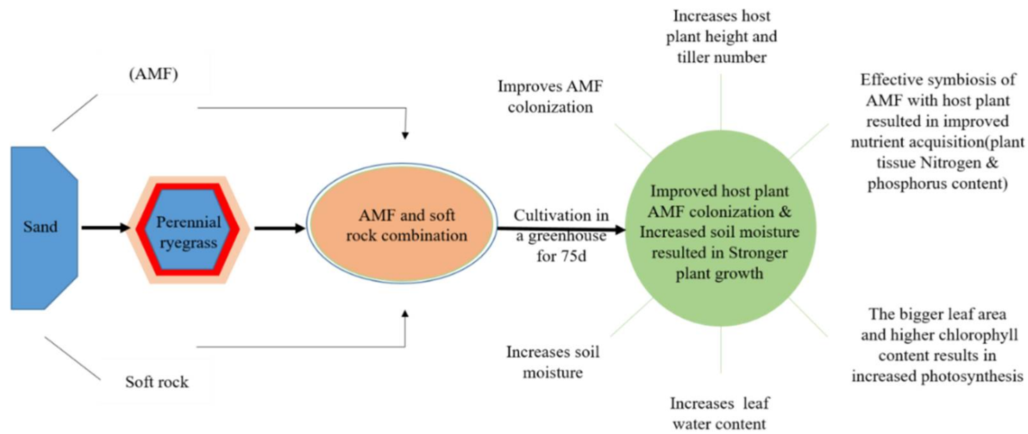

Fig. 5. Improved water holding capacity as well as increased water use efficiency by the combination of SR ameliorator and AMF inoculation improving N and P uptake, relative chlorophyll content as well as photosynthesis, which eventually lead to improved growth performance of perennial ryegrass (*Lolium perenne* L.) under drought condition.

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References

Ahmad H, Hayat S, Ali M, Liu T and Cheng Z 2018. The combination of arbuscular mycorrhizal fungi inoculation (*Glomus versiforme*) and 28- homobrassinolide spraying intervals improves growth by enhancing photosynthesis, nutrient absorption, and antioxidant system in cucumber (*Cucumissativus* L.) under salinity. Ecology and Evolution **8**: 5724-5740.

Bahadur A, Jin ZC, Long XL, Jiang SJ, Zhang Q, Pan JB and Feng HY 2019. Arbuscular mycorrhizal fungi alter plant interspecific interaction under nitrogen fertilization. European J. Soil Biol. **93**: 103094.

Begum N, Ahanger M, Su Y, Lei YA. Mustafa N, Ahmad P and Zhang L 2019. Improved drought tolerance by AMF inoculation in maize (*Zea mays*) involves physiological and biochemical implications. Plants. **8**: 579.

Cheng J, Han JC, Xie JC, Wang HY, Sun ZH, Du YC and Sun YY 2019. The long-term performance of composited soil with feldspathic sandstone amendment on sandy soil and its effects on corn yield. Plant, Soil and Environment **65**(8): 395-400.

Famiglietti JS, Rudnicki JW and Rodell M 1998. Variability in surface moisture content along a hillslope transect: Rattlesnake Hill, Texas. Journal of Hydrology (Amsterdam). **210**(1-4): 0-281.

Fariduddin Q, Mir BA, Yusuf M and Ahmad A 2014. 24-epibrassinolide and or putrescine trigger physiological and biochemical responses for the salt stress mitigation in *Cucumis sativus* L. Photosynthetica **52**(3): 464-474.

Farzaneh M, Vierheilig H, Lössll A and Kaull H 2011. Arbuscular mycorrhiza enhances nutrient uptake in chickpea. Plant, Soil and Environment **57**(10): 465-470.

Fatma M, Asgher M, Masood A and Khan NA 2014. Excess sulfur supplementation improves photosynthesis and growth in mustard under salt stress through increased production of glutathione. Environ. Exp. Bot. **107**: 55-63.

Fixen PE, Grove JH and Westerman RL 1990. Testing soils for phosphorus: soil testing and plant analysis, (third ed), American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin.

Gáborčík N 2003. Relationship between contents of chlorophyll (a + b) (SPAD values) and nitrogen of some temperate grasses. Photosynthetica **41**(2): 285-287.

Han JC, Liu S and Zhang Y 2015. Sand stabilization effect of feldspathic sandstone during the fallow period in mu us sandy land. J. Geogr. Sci. **25**(4: 428-436.

Ma B 2010. Research advances in stress physiological adaptation of perennial ryegrass. Journal of Biol. **7**(2): 58-61.

Miščević P and Vlastelica G 2014. Impact of weathering on slope stability in soft rock mass. J. Rock Mech. Geotech. Eng. **6**(3): 240-250.

Peng B, Han JC, Zhang L and Sun ZH 2019. Effect of feldspathic sandstone on mechanical and mineral composition, water storage and corn (*Zea mays* L.) yield of sand soil in MU US desert, China. Bangladesh J. Bot. **48**(3): 681-687.

Pettersson R and McDonald AJS 1994. Effects of nitrogen supply on the acclimation of photosynthesis to elevated CO_2. Photosynthesis Research. **39**: 389-400.

Rapparini F and Peñuelas J 2014. "Mycorrhizal fungi to alleviate drought stress on plant growth," in use of microbes for the alleviation of soil stresses, ed. M. Miransari (New York, NY: Springer-Verlag).

Rillig MC, Wright SF, Nichols KA, Schmidt WF and Torn MS 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant & Soil **233**(2): 167-177.

Sun ZH and Han JC 2018. Effect of soft rock amendment on soil hydraulic parameters and crop performance in Mu Us Sandy Land, China. Field Crops Res. **222**: 85-93.

Vander Heijden MGA, Boller T, Wiemken A and Sanders IR 1998. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecol. **79**: 2082-2091.

Wahid F, Sharif M and Steinkellner S 2016. Inoculation of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria in the presence of rock phosphate improves phosphorus uptake and growth of maize. Pak. J. Bot. **48**(2): 739-747.

Wang X, Guo Y, Feng X, Shi Y and Duan T 2018. Effect of arbuscular mycorrhiza and a grass endophyte on the drought tolerance of perennial ryegrass. Pratacultural Science **35**(2): 380-390.

Yang Q, Zhao Z, Bai Z, Hou H, Yuan Y and Guo A 2019. Effects of mycorrhizae and water conditions on perennial ryegrass growth in rare earth tailings. RSC Adv. **9**: 10881-10888.

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