Some morpho-physiological characteristic of yellow sweet clover in agri-silviculture system

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Abstract

Drought is one of the most important factors in crop production, which its effects might be compensated by arbuscular mycorrhizal fungi species. Mycorrhizal colonization impacts on the morpho-physiological characteristic of crops. This is done through their role in water and nutrient uptake. Yellow sweet clover (*Melilotus officinalis* L.) belongs to leguminosae family (Fabaceae). This plant has been used for medicinal reasons, providing food and cover for birds and small mammals. Two-year split-plot experiment was conducted based on randomized complete block design with three replications in the plum (*Prunus domestica*) orchard (37º62´N and 44º58´E, altitude 1333 m, West Azerbaijan, Urmia, Iran) in 2016 and 2017. Treatments were mycorrhizal fungi species (*Funneliformis mosseae* and *Rhizophagus irregularis*) and various irrigation systems (rainfed and supplementary). Our results from 2-yr of experiment indicates that the highest (2.21%±0.001) and the lowest (1.84%±0.002) percentage of seed nitrogen were obtained in rainfed system from inoculated (*R. irregularis*) plants in 2nd year and untreated control plants (in both system) in 1st year, respectively. In both systems, we found increase for %leaf and %leaf ratio to aerial parts in mycorrhizal (*F. mosseae*) plants compared to non-AMF inoculated plants in 2nd year. In conclusion, the mycorrhizal plants produced a higher percentage of seed nitrogen, %leaf and %leaf ratio to aerial parts as compared with non-mycorrhizal plants.

Keywords: leaf ratio, mycorrhizal fungi, rainfed, seed nitrogen, supplementary irrigation.

Introduction

Agroforestry systems are based on an arrangement of tree with crop at the same time or successively in the same area, and have as their main plan the optimization of valuable ecological interactions among ecosystem components (Carvalho *et al.,* 2010). Arbuscular mycorrhizal fungi (AMF) are root-colonized soil fungi which shape necessitates symbiotic associations with over 80% of terrestrial plant families (Smith and Read, 2008). These systems have the potential to make the most of the benefits associated with AMF, which sequentially could alleviate negative relations between trees and crops (Carvalho *et al*., 2010). Biennial sweet clover is a winter-hardy, drought tolerant plant that tolerates moderate salinity and grows on a variety of alkaline or slightly acidic soils. This plant is considered high-quality, tasty wildlife and cattle forage (Meyer, 2005). Drought is one of the most important constraints on plant productivity universal and is predictable to increase with climatic changes. Arbuscular mycorrhizal fungi (AMF) can support plant growth, care for host plants from different stresses, and arbitrate plant–plant interactions. AM structure principally promoted sorghum growth, and the biomass and the specific leaf area (SLA) of mycorrhizal plants were larger than those of non-mycorrhizal plants, despite whether they were grown under well-watered or drought stress situation (Sun *et al*., 2017). The increased numbers of leaves found in the AMF inoculated plants are a sign of improve nutritive value (Parissi *et al*., 2007). Mycorrhizae increase growth throughout nutrient uptake. Increased uptake of nutrients by AM fungi is ascribed to the ability of the fungi to move nutrients from soil to the plants away from the "depleting zone" around the roots (Estrada-Luna *et al.*, 2000). AMF provide host plants with about 25% of the whole plant N_2 compared with 3.5% of N_2 supplied by non-inoculated plants (Fulton, 2011). The common purpose of this study was to examination the effects of different AMF on growth characteristics and nutrient supplying (N_2) of yellow sweet clover in relation to different water availability.

Materials and methods

In this study, a 2-year field experiment was executed in the plum (*Prunus domestica*) orchard (37' 62''N and 44' 58''E, altitude 1333m, West Azerbaijan, Urmia, IRAN) during 2016-2017. The experiment was performed in a split-plot arrangement based on a randomised complete block design with three replications. Treatments were two irrigation systems (rainfed and supplementary) as main plot and two AMF species (*Funneliformis mosseae*, *Rhizophagus irregularis* and non-mycorrhizal control) as sub plot. Yellow sweet clover (*Melilotus officinalis*) seeds were collected from fields around Urmia and sown on March 2016 in a silty clay loam soil with a pH 7.84 (measured in 1 M potassium chloride (KCl) with a soil-to-solution ratio of 1:2) and 22.78 mg/kg of phosphorus. The mycorrhizal inoculum (initially isolated from native AMF communities on maize farm) was a combine of sterilized sand, mycorrhizal hyphae and spores (20 spores g-1 inoculum) and colonized root segments that were produced on maize (*Zea mays* L.) host plants by Dr. Y. Rezaee Danesh at

Urmia University, Urmia, Iran. Inoculum was located in the planting rows under seeds and covered with soil. The experimental plots consisted of 6 rows; each row was 3 meters length. The row to row and plant to plant distance was 25 cm and 5 cm, respectively. Supplemental plots were irrigated on 22 May in 2017. In 2016, supplementary irrigation was not necessitating because of rainfall. At the ending of the growing stage, samples were taken from 1.5 m^2 areas in every plot to determine the percentage of leaf, % leaf ratio to aerial parts and %seed nitrogen (seeds, leaves and stem separated). Seed and shoot samples were dried at shade and weighed. Seed nitrogen percentage was estimated with the Kjeldahl method. The digest was watery with distilled water to 100 ml and performed a steam distillation analysis as described by Nelson and sommers (1973). The %leaf and % leaf ratio to aerial parts was ascertained by measuring the leaves, stem and seed dry weight (g/m^2) using the subsequent formulas:

%leaf = leaf dry weight / stem dry weight × 100.

% leaf ratio to aerial parts = leaf dry weight / Biological yield (leaf dry weight + stem dry weight + seed dry weight.

The statistical analyses to figure out the individual and interactive effects were conducted using SAS (Version 9.1.3, SAS Institute Inc., Cary, NC, USA) software. The LSD test was applied to compare the means (at 0.05 probability level).

Result and discussion

Combined ANOVA of 2-year data showed the significant interaction effect of 'Year×Irrigation×Mycorrhizae' on the percentage of seed nitrogen, percentage of leaf and percentage of leaf ratio to aerial part ($P \leq 0.01$) (Table 1). There were significant interaction effect of 'Year×Irrigation' on %seed nitrogen and %leaf ratio to aerial part ($P \leq 0.01$) but not significant on %leaf. At the same time, there were significant interaction of 'Year×Mycorrhizae' and 'Irrigation×Mycorrhizae' on %seed nitrogen, %leaf and leaf ratio to aerial part ($P \leq 0.01$). In addition, the effect of irrigation and interaction of 'Year×Irrigation' on %leaf were not significant (Table 1).

Percentage of Nitrogen

Our results showed that, in two irrigation systems (rainfed and supplementary), there was a significant different between mycorrhizal and untreated control plants in first year. In both irrigation systems, mycorrhizal plants (*F.mosseae* and *R. irregularis)* showed higher %seed nitrogen in comparison of non-mycorrhizal plants (1st year). In second year, there was an increase in $\%N_{seed}$ in mycorrhizal plants (*R. irregularis)* (16.31%) and (13.6%) compared to non-mycorrhizal plants, in rainfed and supplementary systems, respectively. The highest percentage of nitrogen (2.21 ± 0.001) belonged to *R. irregularis* inoculated plants (rainfed system) in 2nd year. The lowest %N (1.84 ± 0.002) was obtained from Non-AMF inoculated plant in 1st year (Table 2).

Percentage of leaf

The results of mean comparison indicated that in both irrigation systems there was no significant different between non-mycorrhizal and *F. mosseae* inoculated plants in %leaf but there was a significant different between Non-AMF inoculated and mycorrhizal (*R. irregularis*) plants in 1st year. Percentage of leaf in untreated control plants was greater than inoculated plants with *R. irregularis*. In second year, the highest %leaf in rainfed system (27.3 ± 0.10) and supplementary irrigation (30.20 ± 0.51) were observed in mycorrhization with *F. mosseae*. In supplementary system, there was no significant different between *R. irregularis* mycorrhizal and untreated control plants. In rainfed system, non-mycorrhizal plants had significantly greater %leaf compared with mycorrhizal plants (*R. irregularis*) (Table 2).

Percentage of leaf ratio to aerial part

In both irrigation systems, Non-AMF inoculated and mycorrhizal (*R. irregularis*) plants had no significant different in %leaf ratio to aerial part in 1st year. Mycorrhization with *F. mosseae* significantly decreased %leaf ratios to aerial part compared to *R. irregularis* mycorrhization. In both systems, as a result, the lowest %leaf ratio to aerial part (43.22 ± 1.70) was obtained from mycorrhizal plants (*F. mosseae*) in first year. In both irrigation systems (2nd year), there were significant different between untreated control plants, *F. mosseae* and *R. irregularis* inoculated plants in % leaf ratios to aerial part .In rainfed system, the highest %leaf ratios to aerial part (51.84 ± 0.30) and the lowest (40.40 ± 0.35) belonged to inoculated plants with *F. mosseae* and mycorrhization with *R. irregularis*, respectively. In supplementary irrigation, there was an increase (38.09%) in %leaf ratio to aerial part in *F. mosseae* mycorrhizal plants compared with non-mycorrhizal plants (second year) (Table 2).

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Table 1- Combined (2-year data) analysis of variance of some morpho-physiological response to mycorrhizal inoculation of yellow sweet clover (*Melilotus officinalis*) under rainfed and supplementary irrigation systems in agroforestry.

Source of variation	df	Mean squares		
		Percentage of seed nitrogen	Percentage of leaf	Percentage of ratio leaf to aerial parts
Year	1	0.123^{**}	7.10^{**}	26.70^{*}
2016	-	1.91b	24.76b	47.40b
2017	-	2.03a	25.64a	49.10a
R(Y)	4	0.0002	0.40	3.10
Irrigation	1	0.0011^{**}	0.70^{ns}	61.85^{**}
Rainfed	-	1.96b	25.10b	46.93b
Supplementary	-	1.97a	25.34a	49.60a
Year×Irrigation	1	0.0011^{**}	0.70^{ns}	61.85^{**}
Error a	4	0.00000003	0.003	0.10
Mycorrhizae	2	0.13^{**}	33.50^{**}	37.10^{**}
Non-AMF	-	1.90c	25.20b	47.81b
F. mosseae	-	1.98b	26.90a	50.20a
R. irregularis	-	2.07a	23.60c	46.73c
Year×Mycorrhizae	2	0.031^{**}	23.90^{**}	338.12^{**}
Irrigation×Mycorrhizae	2	0.004^{**}	2.97^{**}	32.32^{**}
Year×Irrigation×Mycorrhizae	2	0.004^{**}	2.97^{**}	32.32^{**}
Error b	16	0.000032	0.20	18.20
Coefficient of variation (%)		0.30%	1.60%	2.21%

ns, not significant; * and ** significant at the 5 and 1% probability level, respectively.

Table 2- comparison of 2-year means of yellow sweet clover (*Melilotus officinalis*) physio-morphological traits by rainfed and supplementary irrigation and mycorrhizal fungi species.

Year	Irrigation	Mycorrhizae	Percentage of seed nitrogen	Percentage of leaf	Percentage of leaf ratio to aerial part
2016	Rainfed	Non-AMF	1.84±0.002	25.60±0.10	49.42±0.30
		F. mosseae	1.95±0.013	24.83±0.80	43.22±1.70
		R. irregularis	1.95±0.010	23.90±0.32	49.50±1.62
	Supplementary	Non-AMF	1.84±0.002	25.60±0.10	49.42±0.30
		F. mosseae	1.95±0.013	24.83±0.80	43.22±1.70
		R. irregularis	1.95±0.010	23.90±0.32	49.50±1.62
2017	Rainfed	Non-AMF	1.90±0.001	25.45±0.15	47.25±0.40
		F. mosseae	1.98±0.002	27.73±0.10	51.84±0.30
		R. irregularis	2.21±0.001	22.92±0.16	40.40±0.35
	Supplementary	Non-AMF	1.90±0.002	23.96±0.50	45.20±1.10
		F. mosseae	2.06±0.001	30.20±0.51	62.42±1.50
		R. irregularis	2.16±0.001	23.61±0.22	47.60±0.82

Values are means ± SD obtained from three replications.

Discussion

Drought induces morphological and physiological changes that adversely affect plant growth and yield through diminishing leaf area and accelerating leaf senescence. Plant capability to absorb and distribute nutrients is a main factor in plant tolerance to drought. Arbuscular mycorrhiza fungi improve the growth and development of colonized plants. The root colonization by the mycorrhiza enhance active absorptive surface area and encourage water uptake even in water stress situation, it could increase the drought tolerance by means of increasing soil water movement to the plant roots. At the same time, AMF enhancing the permeability cells of root, causing an increase in water absorption and nutrients uptake mainly phosphorous and micronutrients (Artursson *et al*., 2006). Our finding in two irrigation systems (rainfed and supplementary) showed improved

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%N $_{seed}$ of yellow sweet clover to mycorrhization with AMF (Table 2). Considering all given information, there is an increase in %leaf and %leaf ratio to aerial part in mycorrhizal (*F. mosseae*) yellow sweet clover, especially in supplementary system (second year). AMF improvement could be affected not only by soil water content but also by soil factors. Both irrigation and mycorrhizae application affected significantly the percentage of arbuscules in the root system. Under limited irrigation the percentage of arbuscules in the root system reduced (Apostolos *et al*., 2017).

Conclusion

This study supplied proof that AMF have the potential to be helpful on the growth of yellow sweet clover (*Melilotus officinalis)* under rainfed and supplementary systems. Because of the effects of mycorrhizae application to host plant are complex, additional studies on *Melilotus officinalis*–AMF symbiosis in the agroforestry system are needed.

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