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Effect of arbuscular mycorrhiza and phosphorus levels on growth and water use efficiency in Sunflower at different soil moisture status

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A pot experiment was carried out to study the effect of mycorrhizal associations on the growth of sunflower under moisture stress conditions at three levels of soil phosphorus. Mycorrhizal plants showed high leaf area, leaf area duration and total dry matter at 100% field capacity (FC) and 50 mg P/kg oil. The cumulative water use and water use efficiency (WUE) was also high in mycorrhizal plants. WUE increased with the P level and reduced with moisture stress. Stomatal conductance and transpiration rate was high in mycorrhizal plants. Phosphorus levels also influenced stomatal conductance and transpiration rates. Both mycorrhizal plants and high soil P levels increased partitioning of biomass more towards root, resulting in higher root to leaf weight ratio. The plants with mycorrhizal associations had low stomatal and mesophyll limitations for photosynthesis.

Key words: mycorrhizal plants, non-mycorrhizal plants, water use efficiency, cumulative water transpired, stomatal and mesophyll conductance

Introduction

A primary limitation of crop production in semi arid region is the deficiency of available nutrients especially phosphorus and water. In this region, sunflower is grown mainly under rain fed condition where drought may occur at any time during the growth season. Any input, which enables the plants to withstand drought stress, would help to improve crop production.

Arbuscular Mycorrhizal (AM) fungi associated with plant roots were found to enhance productivity under drought conditions by improving the mineral nutritional status, mainly phosphorus (P) (Al-Karaki and Al-Raddad, 1997). In addition, AM symbiosis may alleviate plant responses to moderate

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moisture deficiency by several mechanisms including increased water uptake due to extraction of water in the soil by hyphae (Davies *et al*., 1992, Ruiz-Lozano *et al*., 1995, Pai *et al*., 1994), altered hormonal levels causing changes in stomatal conductance (Druge and Schonbeck, 1992), increased turgor by lowering leaf osmotic potential (Davies *et al*., 1992) and improved maintenance of the soil root continuum (Sweatt and Davies, 1984). Goicoechea *et a.l* (1995) suggested the importance of AM symbiosis in maintaining cytokinin levels under drought.

The symbiotic interaction between AM fungi and host plants grown under drought conditions especially at different levels of P needs to be studied in order to optimize beneficial effects of AM fungi. This present study was done to determine the effects of AM fungal inoculation at different levels of P on growth and water use efficiency in sunflower under different soil moisture levels.

Materials and methods

A pot experiment was conducted to study the effect of moisture stress on growth and water relations of the mycorrhizal and non- mycorrhizal sunflower plants. The plants were grown in plastic pots holding 5 kg of steam-sterilized soil. The soil used was an alfisol of the type fine kaolinitic isohyperthermic typic kanhaplustalfs with pH 5.5 and an available P of 1.8 kg/ha. Recommended N, P, K and micronutrients were applied. KH_2PO_4 as a source of phosphorus was used at three different levels. They are control (without P), 50 mg of P/kg of soil and 100 mg P kg/soil. For half the number of pots, 20 g of mycorrhizal inoculum (*Glomus fasciculatum*) was placed 2 cm below the seeding spot and the other half numbers of pots were uninoculated. Three seeds were sown in each spot and thinned to one after 12 days. Plants were maintained in a glass house.

A nutrient solution containing KCl, $MgSO_4.7H_2O$, $CuSO_4.5H_2O$, H_3BO_3, $NaMoO_4.2H_2O$, $ZnSO_4.7H_2O$ and Mg $(NO_3)_2.$ $6H_2O$ was added to the sterilized soil at the rate of 100, 50, 10, 0.5, 10 and 34.6 mg of K, Mg, Cu, B, Mo, Zn and N per kg of soil.

The moisture stress treatment was given by bringing the soil water status to 100%, 70% and 50% field capacity (FC). For maintaining the soil water status at 70% and 50% FC, pots with plants were allowed to dry to the required level. After each day the pots with the plants were weighed in the morning hours and the amount of water lost by evapotranspiration (ET) from the soil was replaced by watering the pots with the same volume of water to bring the soil water status back to 100, 70 and 50% FC. The soil surface in each pot was

covered with thick plastic sheet to reduce direct evaporation of water from the pot surface. A set of five pots each with soil and the plants were maintained at 100, 70, 50 % FC to know the direct evaporation of water from the pot surface. These pots were also weighed daily (Evaporation loss E) and moisture status of the soil was brought back to required level. From this basic value of water applied, cumulative water transpired (CWT) for 28 days of experimental period from each pot was computed (ET-E=CWT). Each main treatment had three levels of moisture with nine replications. These treatments were imposed for 28days. The plants were harvested 57 days after sowing. Observations on leaf area were recorded just prior to the addition of water to the pots and also an hour after the addition of water.

Initial biomass and leaf area (cm^2/plant) before imposing moisture stress treatments was determined in a set of 5 containers representing each level of phosphorus on 28 DAS. Final biomass and leaf area were once again recorded at the end of the experiment (57 DAS). From these values total biomass accumulated during the experimental period and the amount of biomass produced per unit of water i.e., water use efficiency (WUE) was computed as follows:

WUE (g dw/kg H_2O) = (BM 28 - BM 57) / CWT

Where, BM 28 is the total dry matter (g/plant) on 28 days and 57 days, respectively.

The leaf area was determined by using leaf area meter and leaf area duration (LAD), the functional leaf area during the experimental period, was calculated as follows:

LAD (dm^2/day) = [(LA 28 + LA 57) / 2] x 28

Where, LA 28 and LA 57 are the leaf area on 28 and 57 days, respectively.

Results

Mycorrhizal inoculum increased the leaf area of the plant by 14.3%. Phosphorus nutrition was efficient in influencing the leaf area development in sunflower on 30 DAS. Maximum leaf area was observed in plants, which received 100 mg P/kg of soil.

Moisture stress resulted in marked reduction in leaf area. The leaf area per plant decreased by 19.27% and 19.6% at 75% and 60% FC compared to plants which were maintained at 100% FC. At harvest the reduction in leaf area due to moisture stress was to an extent of 22.8% and 32.13% at 75% and 60% FC.

The LAD was more in mycorrhizal plants to an extent of 7.1%. There was an increase in 6.7% and 18.5% LAD at 60 and 100 mg P/kg of soil

compared to control. Moisture stress resulted in drastic reduction in LAD in plants maintained at 75 and 60% FC.

The amount of biomass accumulated in mycorrhizal plants was significantly more than the non-mycorrhizal plants. Mycorrhizal plants accumulated about 99.72% more biomass during the experimental period. The P effect on biomass accumulation was highly significant when the soil had 100 mg P/kg of soil, registering 16.2% increased biomass over "0" level of P. The biomass accumulation drastically reduced with moisture stress resulting in 28.01% and 35.75% reduction at 75 and 60% FC respectively (Table 1).

Table 1. Leaf area (cm^2 / plant), leaf area duration (dm^2 /day), final dry matter (g) and root to leaf weight ratio (w/w) as affected by mycorrhiza, phosphorus level and moisture level.

Treatments		Leaf area		LAD		Final DM		Root to leaf weight ratio	
Field capacity	**P-level**	**NM**	**M**	**NM**	**M**	**NM**	**M**	**NM**	**M**
100%	**P0**	622.45	710.86	98.26	109.75	6.83	7.74	0.49	0.99
	P1	688.90	738.74	106.89	113.38	7.14	9.07	0.54	1.57
	P2	718.35	852.58	110.55	128.17	7.31	9.43	0.79	1.28
75%	**P0**	512.53	489.92	83.97	81.03	5.04	6.31	0.48	0.83
	P1	593.59	514.74	94.51	84.25	5.23	6.47	0.41	1.21
	P2	604.07	626.47	96.01	98.77	6.00	7.06	0.59	1.06
60%	**P0**	434.60	423.63	73.84	72.41	4.58	5.87	0.39	0.68
	P1	437.90	500.94	73.34	82.46	4.78	6.223	0.39	0.90
	P2	525.87	616.98	85.70	97.55	5.05	6.44	0.86	1.11

NM = Non-mycorrhizal plants
M = Mycorrhizal plants
P0 = Control
P1 = 50 mg P/kg soil
P2 = 100 mg P/kg soil

LSD at 5%
Mycorrhiza : 0.16
P-level : 0.19
Moisture level: 0.19

Cumulative water transpired (CWT) by the plant i.e. the amount of water used for 28 days per plant differed significantly between mycorrhizal and non-mycorrhizal plants. At P levels of 50 and 100 mg P/kg of soil, CWT increased by 5.3% and 11.37% respectively compared to control without P. The amount of total water used during the experimental period was significantly less in plants maintained at 100% FC than those maintained at 75% and 60% FC. The water used by the mycorrhizal plants was 20.46% more compared to non-mycorrhizal plants.

The amount of biomass produced per unit of water (WUE) was higher in mycorrhizal plants by 20.52% compared to non-mycorrhizal plants. P levels

also influenced WUE. This also increased with increase in P levels. However, WUE decreased under moisture stress. At 75% and 60% FC, WUE values decreased by 3.34% and 12.38% respectively compared to 100% FC. WUE was high in mycorrhizal plants at all levels of P and at different levels of soil water status (Table 2).

Table 2. Influence of mycorrhiza, P-level and moisture level on cumulative water transpired (CWT) (ml/plant) and water use efficiency (WUE) (g dw/kg H_2O).

Treatments		CWT		WUE	
Field capacity	**P-level**	**NM**	**M**	**NM**	**M**
100%	**P0**	2049.0	2037.0	2.82	3.28
	P1	2142.4	2356.0	2.84	3.40
	P2	2204.0	2392.6	2.84	3.50
75%	**P0**	1487.0	1623.0	2.68	3.24
	P1	1533.0	1652.4	2.72	3.28
	P2	1804.4	1765.0	2.74	3.40
60%	**P0**	1409.0	1681.6	2.50	2.86
	P1	1478.0	1681.2	2.52	3.08
	P2	1572.8	1712.4	2.54	3.12

NM= Non-mycorrhizal plants M= Mycorrhizal plants

LSD at 5%

Mycorrhiza: 108.06 Mycorrhiza : 0.23

P-level: 132.35 P-level: NS

Moisture level: 132.35 Moisture level: 0.28

Stomatal conductance was recorded 20 days after imposition of the stress treatment and was measured three hours after bringing the moisture to desired FC. Observations indicated that mycorrhizal plants maintained higher stomatal conductance to an extent of 24.76% compared to non-mycorrhizal plants (Table 3). P levels did not influence the stomatal conductance. However, plants maintained at 100% FC showed relatively high stomatal conductance.

Conductance measured prior to watering indicated that it is significantly less in mycorrhizal plants compared to non-mycorrhizal plants. Stomatal conductance values determined at an hour after watering indicated that mycorrhizal plants showed marginally higher conductance over non-mycorrhizal plants. Even after watering, the stomatal conductance was relatively less in plants maintained at 75 and 60% capacity (Table 3).

Table 3. Effect of mycorrhiza, P-level and moisture level on stomatal conductance (m mol/m^2/sec) before and after watering the plants.

Treatments		Before watering		After watering	
Field capacity	**P-level**	**NM**	**M**	**NM**	**M**
100%	P0	690	620	720	665
	P1	720	490	680	550
	P2	600	720	535	675
75%	P0	500	590	515	525
	P1	530	575	550	530
	P2	505	510	540	540
60%	P0	530	500	620	580
	P1	340	315	535	595
	P2	665	345	610	690

NM = Non-mycorrhizal plants
M = Mycorrhizal plants

Transpiration rate observed 20 days after imposition of stress was 20.23% more in mycorrhizal plants. Transpiration rate did not show any trend in relation to P concentration although a marginal increase was observed at 100% FC. At one hour after irrigation, transpiration rate was marginally high in mycorrhizal plants (Table 4).

Table 4. Effect of mycorrhiza, P-level and moisture level on transpiration rate (ug/cm^2/sec) before and after watering the plants.

Treatments		Before watering		After watering	
Field capacity	**P-level**	**NM**	**M**	**NM**	**M**
100%	P0	15.09	20.94	21.31	19.37
	P1	19.75	21.86	19.85	21.31
	P2	21.05	21.15	20.90	21.52
75%	P0	14.17	18.61	18.61	18.26
	P1	18.71	17.95	17.84	17.65
	P2	16.67	16.85	17.20	16.93
60%	P0	20.91	19.45	20.48	19.64
	P1	20.92	15.04	19.24	18.31
	P2	20.69	19.61	18.53	14.12

NM = Non-mycorrhizal plants
M = Mycorrhizal plants

Discussion

Mycorrhizal association improved the LAD, as well as functional LA, indicating that the vegetative growth of the plant and in particular, the total functional photosynthetic surface area improved in the presence of mycorrhizal

association. P level also influenced LAD and total functional leaf area indicating the importance of P nutrition on the development of leaf area. Influence of mycorrhizal association on improving the leaf size and LAD has been reported by number of workers. (Panwar, 1993; Dixon, 1994).

The biomass accumulation also showed significant increase with the mycorrhizal association. Similarly soil P and soil water content also influenced the biomass accumulation during the experimental period. At all the levels of soil P and moisture status, inoculated plants had higher biomass accumulation. At "0" level of P in the soil, the mycorrhizal plants had 22.58% higher biomass compared to non-mycorrhizal plants, while at 50 and 100 mg P/kg soil mycorrhizal association resulted in higher biomass accumulation of 12.3% and 2.01% respectively. This indicates the importance of mycorrhizal association at low available soil P condition. Significant growth enhancement at low available soil P has been observed in different plant species (Johnson and Hummel, 1985; Bethlenfalvey *et al*, 1988a; Osunubi *et al*., 1992).

Mycorrhizal association as well as high P soil levels increased partitioning of biomass more towards root, resulting in high root to leaf weight ratio. Although moisture stress reduced the partitioning of biomass to leaf, at all the levels of soil moisture status mycorrhizal plants showed high root to leaf ratio. Earlier studies have indicated that mycorrhizal root system has higher branching as well as higher partitioning of biomass to root (Osunubi *et al*., 1992; Bethlenfalvey *et al*., 1988a).

The total amount of water used by the plant and amount of biomass accumulated per unit amount of water showed that mycorrhizal plants used significantly more water during the experimental period. The development of more roots and the requirement of more water to sustain high growth rate might have influenced more water use by mycorrhizal plants.

A careful analysis of the total amount of water used by the plant and amount of biomass accumulated per unit amount of water used by the plant showed that mycorrhizal plants used significantly more water during the experimental period. On an average, the water used by the mycorrhizal plants was 20.46% more compared to non-mycorrhizal plants. The development of more roots and the requirement of more water to sustain high growth rate influenced more water use by mycorrhizal plants. Other workers have also reported higher water use by mycorrhizal plants compared to non-mycorrhizal plants (Allen *et al*., 1981; Hardie and Leyton, 1981). High shoot water status in mycorrhizal plants compared to non-mycorrhizal plants during drought has been reported by some workers (Subramanian *et al*., 1997).

The WUE, an indicator of the amount of biomass produced per unit of water used was significantly high in inoculated plants. The amount of water

lost per unit LAD did not differ between mycorrhizal and non-mycorrhizal plants. However, P levels influenced the rate of water use with increase in P nutrition and there was an increase in rate of water loss, suggesting mycorrhizal association not only influences the water uptake but also helps in high biomass synthesis per unit amount of water used probably by maintaining high photosynthetic rate per unit LA.

Mycorrhizal plants always showed higher stomatal conductance and transpiration rate. P level also influenced stomatal conductance and transpiration rate. This is in conformity with the observations made earlier in cowpea and citrus (Duan *et al*., 1996 and Fidelibus *et al*., 2001). The present study brings out that higher water consumption in mycorrhizal plants is because of high transpiration rate, low stomatal conductance and increased water uptake by roots compared to non-mycorrhizal plants. At low soil phosphorus, enhanced growth and water relations in mycorrhizal plants may be due to better phosphorus nutrition. At high phosphorus, improved plant growth and water relations in mycorrhizal plants can be attributed to better water consumption and water utilization.

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