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Growth, Water Use Efficiency and Antioxidant Defense Responses of Mycorrhizal and Non Mycorrhizal Allium sativum L. Under Drought Stress Condition

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Abstract: Garlic plant inoculated with Arbuscular Mycorrhizal (AM) fungi Glomus fasciculatum showed improved plant growth shoot length and biomass during drought stress condition. The mycorrhizal garlic plants were absorbing more water during drought stress condition by extraradical hyphae which were spread in soil, which helps in increasing water use efficiency of mycorrhizal garlic plants. The antioxidant defense response either increase or decrease due to response of drought stress. Proline accumulation in shoot was induced by drought stress in non AM inoculated garlic plants. But in root, proline accumulation was higher in AM garlic plants. This suggests that AM fungi help the garlic plants to greater osmotic adjustment under drought stress condition. The work suggest that AM fungi helps the garlic plants by absorbing more water and improved plant biomass by minimizing drought stress effect also activating the antioxidant and non antioxidant defense system to withstand under drought condition.

Keywords: Allium sativum L., Water Use Efficiency; Drought; AM fungi; Antioxidant; Proline.

INTRODUCTION

Arbuscular mycorrhiza (AM) fungi symbiotically associated with plant roots are known to enhance crop productivity under drought conditions by improving the mineral nutritional status (mainly P) (1), in addition other factors associated with AM fungal colonization may influence plant resistance to drought. These include, increase in root length and depth, development of external hyphae (2) increasing transpiration rate and lowering of stomatal resistance (3); increasing water turgour potentials (4). The interaction between roots and at the same time nourishment the AM has been described in terms of Benefit/cost analysis (5).

Activated oxygen species (AOS) such as superoxide (O_2^-), H_2O_2 and hydroxyl radicals (OH^-) are formed as byproducts of normal metabolism in different cellular organelles (6) Under drought stress condition an increase in generation of AOS has been described (7) that may cause damage initiated by AOS. Plants possess several mechanisms that detoxify O_2^- and H_2O_2 called antioxidant system. The components of antioxidant defense system include enzymes such as Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT) etc. It has been reported that inoculation with AM produces an increase in some antioxidant enzymes in shoots from mycorrhizal shrub species deforested in a degraded semi-arid soil (8) and roots in most of mycorrhizal soybean plant (9). In mycorrhizal inoculated citrus plant the increased POD activity and reduced CAT activity was observed as compared with non mycorrhizal plant (10).

Garlic (Allium sativum L.) has been known as folk medicine, In the year of 2010 garlic is one of the crops growing in 209.34 thousand hector area of arid and semiarid regions of India and production is 1264.69 thousand metric ton (Data Source: Directorate Of Economics and Statistics, Krishi Bhawan, New Delhi, India). It's an important bulb crop widely used as a spice/condiment; medicinally garlic is used against arteriosclerosis, high blood pressure, and has been shown to have antibacterial, antifungal, antiviral and antiprotozoal activities. It also modulates the cardiovascular and immune system and has antioxidative and anticancerogenic properties (11). India is second largest country after China in garlic cultivation, but the productivity will be less than in other countries. Allium species including garlic are responsive to AM symbiosis. In absence of AM fungi onion growth is stunted (12).

The main objective of this study is to see effect of AM on garlic plants with respect to increase in growth and understand their role in withstanding drought stress condition.

MATERIALS AND METHODS

Plant material and experimental design:

The cloves of Garlic (Allium sativum L.) variety named Godavari (selection 2) were surface sterilized with 0.01% $HgCl_2$ and grown in plastic bags containing 1.5 kg steam sterilized (121^{0}C and 1056 g/cm2 pressure for 1 h, autoclaved twice) garden Sandy loamy soil. The mycorrhizal inoculum was grown on Guania grass in autoclaved soil. T Three-month old 30 g of mycorrhizal inoculum G. fasciculatum (Thaxt.) Gerd. & Trappe containing AM colonized roots, rhizosphere soil having extramatrical mycelium and spores (10-15 spores/g) were placed below the seeds in small trench (Adholeya et al., 2005). The experimental design consist of total of 96 plants were arranged in a completely randomized block design (CRBD) with 8 replicate plants per treatment. The treatments were 1) non-GF treated well watered (Cww) 2) non-GF treated drought stress treatment (Cdt) 3) GF

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treated well watered (GFww) 4) GF treated drought stress treatment (GFdt). The experiment was conducted in natural light. This was watered daily for 21 days until drought treatment was initiated by withholding of water.

Determination of Growth Performance:

The growth performance was observed with respect to plant fresh and dry weight, shoot and root length, Water Use efficiency (WUE), Benefit for plant with AM-root association were calculated according to the following formulae (13).

WUE= water use efficiency (g DM kg^{-1} evapotranspired water)

The roots were analyzed by clearing and staining the roots using the (14) method and percentage of AM root colonization was determined using the gridline intersect method (15).

Enzyme extracts were prepared in extraction buffer containing 1 M Tris acetate buffer, pH 6.0, 0.5 M Na2$EDTA (pH 8.0), 2% w/v PVP, 0.1 mM PMSF., 0.2% v/v Triton X 100 by grinding 0.5 g leaf or root sample in 3 ml extraction buffer, extracts were centrifuged at 10,000g at 4^oC for 10 min and the supernatant was used as enzyme source. The SOD, POD and CAT enzymes were estimated using the methods previously described by (16, 17 and 18), respectively. Protein was estimated as described by (19). The percentage increment was calculated as treatment minus control, divided by control and multiplied by 100. Non-enzymatic response, such as proline accumulation, was estimated using the method as described by (20). Three plants were taken from each treatment for analysis.

Statistical analysis:

Statistical analyses were performed Two way ANOVA for shoot length, plant fresh weight and water use efficiency. For antioxidant and non antioxidant enzymes, one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) were done. The values are mean ± SD for * p≤0.05 was considered significant.

RESULTS

Morphological parameters:

As shown in Table. 1, Shoot length showed significant increase in AM inoculated plants after 8^{th} days of drought period. After 16^{th} day of drought period mycorrhiza as well as drought stress significantly affecting shoot length of garlic plants (Table 1). Plant fresh weight significantly affected by AM after 8^{th} day of drought period and both AM as well as drought stress was affecting the fresh weight after 16^{th} day of drought stress (Table 2).

The root colonization in GF well watered garlic plants was (30% and 63.33%) slightly higher percentage of AM colonization as compared to GF drought stress Garlic plants (20% and 58.33%) in both after 8^{th} and 16^{th} day of drought period. As shown in table.3, after 8^{th} days of drought period WUE was significantly affected by AM fungi but not by drought. After 16^{th} day of drought period WUE was significantly affecting by AM fungi as well as drought period. Mycorrhizal colonization significantly improved WUE in the water- stressed garlic plants but the values remained lower as compared to the values for well-watered garlic plants in all cases. Mycorrhizal colonization did not significantly affect most parameters in well watered garlic plants, but did improve WUE during 8^{th} day of drought period. But after 16^{th} day of drought period Water use efficiency was highest in the GF well watered treated garlic plants and lowest in GF drought treatment. WUE in the GF drought stress treatment was significantly higher than non-inoculated well watered treated garlic plants (Table 3). The calculated Benefit values for Garlic plants in response to AM inoculation was (0.048 gm/plant dry weight) found to be lower after 8^{th} day of drought period, but after 16^{th} day of drought period it went on increasing under water-stressed conditions (0.125 gm/plant dry weight).

Table.1: Effect of AM fungi and drought stress on shoot length of Mycorrhizal (AM) and non-mycorrhizal Allium sativum L. after 8th and 16th day of drought stress condition: Two way ANOVA of factorial design.

Treatments		8^{th} day	16^{th} day
Non-AM	Cww	21.83 ± 0.235 b	27.50 ± 0.979 a
	Cd	20.50 ± 2.677 b	17.00 ± 3.559 b
AM	AMww	26.23 ± 1.020 a	32.43 ± 1.552 a
	AMd	28.50 ± 1.471 a	26.83 ± 2.778 a
Significance(a)	AM	*	*
	Drought	ns	*
	AM X Drought	ns	ns

Value of *P≤ 0.05 are significant

Table.2: Effect of AM fungi and drought stress on plant fresh weight of Mycorrhizal (AM) and non-mycorrhizal Allium sativum L. after 8th and 16th day of drought stress condition: Two way ANOVA of factorial design.

Treatments		8^{th} day	16^{th} day
Non-AM	Cww	0.828±0.092 b	1.682±0.175b
	Cd	0.848±0.074b	0.43±0.150c
AM	AMww	1.848±0.192a	2.620±0.419a
	AMd	1.161±0.380b	1.189±0.305b
Significance(a)	AM	*	*
	Drought	ns	*
	AM X Drought	ns	ns

Value of *P≤ 0.05 significant

Table.3: Effect of AM fungi and drought stress on Water use efficiency (WUE) of Mycorrhizal (AM) and non-mycorrhizal Allium sativum L. after 8th and 16th day of drought stress condition: Two way ANOVA of factorial design.

Treatments		8th day	16th day
Non-AM	Cww	0.649±0.078b	1.455±0.207 b
	Cd	0.713±0.087 b	0.312±0.151 c
AM	AMww	1.533±0.138 a	2.261±0.370 a
	AMd	0.985±0.399 b	0.946±0.283 b
Significance(a)	AM	*	*
	Drought	ns	*
	AM X Drought	ns	Ns

Value of *P≤ 0.05 are significant
*Means within a column followed by the same letter are not significantly different (P ≤ 0.05).

Biochemical parameters:

Shoot and root POD activity in mycorrhizal drought stressed garlic plants was found to increase significantly after 16th day of drought period. POD activity in shoot of garlic plants was found to be lower as compared to the roots (Table no 4 and 5).

Table.4: Antioxidant enzyme activities in shoot of (AM) and non-mycorrhizal Allium sativum L. after 8th and 16th days of drought stress condition.

Treatments	Catalase ($U\ min^{-1} mg^{-1}$ protein)		Peroxidase Unit $min^{-1} mg^{-1}$ protein		Superoxide dismutase Unit/h/mg protein	
	8th day	16th day	8th day	16th day	8th day	16th day
C ww	3.057±0.129c	0.197±0.020c	0.225±0.032b	0.251±0.054b	0.309±0.051b	0.037±0.020c
Cd	4.478±0.409b	0.122±0.032c	0.366±0.039a	0.426±0.052a	0.549±0.051a	0.186±0.096ab
Gfww	4.280±0.251b	0.669±0.064b	0.141±0.019c	0.282±0.035b	0.295±0.038b	0.151±0.025bc
Gfd	8.478±0.423a	0.807±0.080a	0.216±0.025b	0.451±0.042a	0.551±0.145a	0.291±0.037a

*Means within a column followed by the same letter are not significantly different (P ≤0.05).

Table.5: Antioxidant enzyme activities from roots of (AM) and non-mycorrhizal Allium sativum L. after 8th and 16th days of drought stress condition.

Treatments	Catalase ($U\ min^{-1} mg^{-1}$ protein) *		Peroxidase Unit $min^{-1} mg^{-1}$ protein *		Superoxide dismutase Unit/h/mg protein*	
	8th day	16th day	8th day	16th day	8th day	16th day
C ww	1.054±0.238c	0.482±0.082c	0.064±0.045c	0.524±0.106b	0.118±0.066c	0.251±0.092a
Cd	11.432±0.775b	1.224±0.071a	0.323±0.082b	0.526±0.094b	0.302±0.053b	0.277±0.013a
Gfww	12.356±0.537b	1.064±0.046b	0.408±0.057b	1.630±0.488a	0.221±0.022bc	0.287±0.023a
Gfd	22.510±0.646a	1.370±0.045a	1.136±0.156a	1.823±0.440a	0.548±0.069a	0.373±0.115a

*Means within a column followed by the same letter are not significantly different (P ≤ 0.05).

After 8th day of drought period the shoot SOD activity was found to increase significantly in GF drought stress garlic plants as compared to well watered garlic plants (Table no 4). After 16th day of drought period, shoot SOD activity was found to be significantly higher in non mycorrhizal drought garlic plants than non mycorrhizal well watered plant.

In GF drought treated garlic plants, root SOD activity after 8th day of drought period showed significant increase as compared to other treatments. Root SOD activity in non mycorrhizal drought stressed garlic plants showed higher SOD activity than non mycorrhizal well watered garlic plants, but it was not significantly different as compared to GF well watered treatment (Table no 5).

After 8th day of drought period, shoot CAT activity in GF drought stressed garlic plants was found to be significantly higher. After 16th day of drought period, shoot CAT activity in GF well watered and drought treated garlic plants increased significantly as compared to non-mycorrhizal garlic plants (Table no 4). Root CAT activity, after 8th day of drought period was found to be more in GF drought treated garlic plants. In non-mycorrhizal drought stressed garlic plants, CAT activity was found to be more as compared to non mycorrhizal well watered garlic plants. After 16th day of drought period, there was not much difference in the activity of CAT in all the specified treatments (Table no 5).

The shoot Proline accumulation in mycorrhizal plant was significantly less as compared to non mycorrhizal plant after 8th day of drought period. After 16th days of drought period, shoot proline accumulation in GF inoculated drought stress garlic plants increased significantly as compared to non mycorrhizal garlic plants (Figure 1). Proline accumulation in roots after 8th day of drought period was found to be more in non mycorrhizal drought stress garlic plants than other treatments. Proline accumulation in roots of GF inoculated well watered condition was found to be lower than other treatments. After 16th days of drought period, GF inoculated drought stress garlic plants showed significantly higher amount of proline accumulation than other treatments (Figure 2).

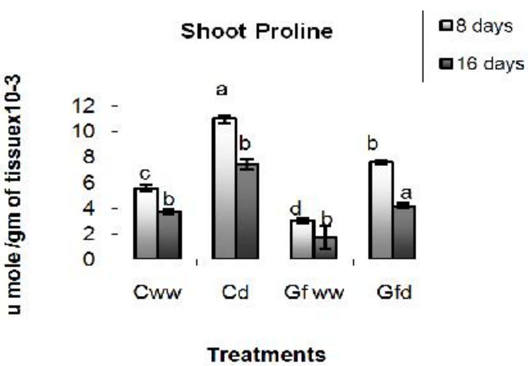

Figure.1: Shoot Proline accumulation of Mycorrhizal (AM) and non-mycorrhizal Allium sativum L. grown with and without drought stress after 8th and 16th day of drought stress condition.

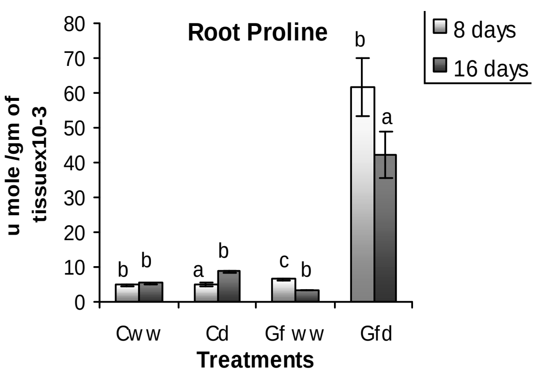

Figure.2: Root Proline accumulation of Mycorrhizal (AM) and non-mycorrhizal Allium sativum L. grown with and without drought stress after 8th and 16th day of drought stress condition.

DISCUSSION

Under drought stress condition mycorrhiza inoculated Garlic plants enhanced the growth in terms of plant fresh. This enhanced growth was due to the mycorrhizal inoculation has been attributed to improved water relations resulting from enhanced P nutrition (21). Mycorrhizal fungi enhance shoot length by improving water relation in garlic plants. It is clear that the AM fungi inoculation is an effective measure to enhance drought resistance in Garlic plant. From the results of mycorrhizal colonization, the AM colonization was not affected by drought stress. The mycorrhizal garlic plants utilized less water to produce one unit of shoot DM (i.e. higher WUE) than non-mycorrhizal garlic plants, but water stressed and well-watered garlic plants did not differ in WUE. (22). Higher WUE in mycorrhizal than non-mycorrhizal garlic plants may indicate that AM increased the ability of roots to absorb soil moisture, thus maintaining opened stomata in leaves and enhancing DM production. Enhanced water conductivity has been attributed to increased area for water uptake provided by AM hyphae in soil (23). Responses of the host plant, AM, and soil condition to AM symbiosis may be useful criteria for selecting "efficient" plants for water-stress conditions. These responses have sometimes been discussed in terms of "Benefits" to plants (13). For the AM-root symbiosis seems to be beneficial, plants colonized with AM should have less biomass loss and produce more DM than non-mycorrhizal plants when grown under water stress (24). The AM hyphae absorb mineral nutrients, especially P and water, which benefits the host plant. In turn, the host plant supplies carbohydrates and energy to the AM. The AM may thus act as respiratory and growth sinks and drain host plant resources (13), The calculated Benefit values of GF inoculated Allium sativum L. plant was found to be more in water-stressed conditions than under well-watered conditions.

Drought causes an oxidative stress in plants and many of the degenerative reactions associated with abiotic stresses are mediated by reduced oxygen species such as O_2^- and H_2O_2 (25). In present study focus has been made on the activity of antioxidant enzymes, like role of SODs.

Little attention has been paid to the role of other important antioxidant enzymes such as CAT, POD during drought stress condition inoculated with AM fungi. Plants possess hydrogen peroxide scavenging enzymes: POD and CAT. Detoxification of the reactive oxygen protects cell against harmful concentration of hydroperoxides (26Castillo 1992). Different responses in mycorrhizal plants have been observed frequently. The POD activity in the shoots of garlic plants was found to be more under non mycorrhizal drought stressed conditions. Root POD activity was higher in GF inoculated drought stressed garlic plants. Higher POD activity protects plants against the oxidative stresses (27). Root SOD activity of GF drought stressed condition was significantly higher. SOD plays a role in detoxification processes by catalyzing the conversion of free O_2^- to O_2 and H_2O_2, very often is associated with stress situation including plant/pathogen interaction (21). Higher levels of SOD activities were observed in lettuce (Lactuca sativa) roots colonized by Glomus mosseae or Glomus deserticola under drought stress (28) and this response is at the transcriptional level (29).

Proline is an indicator of drought tolerance in drought stressed plant tissue as proline accumulation helps to maintain high osmotic levels in plant cells suffering from water deficit (30). In our experiment shoots of GF inoculated garlic plants also showed higher concentrations of proline than Cd plants during drought stress, which was also attributed by the greater drought resistance of AM plants, i.e. more effective osmotic adjustment (31). Higher proline accumulation in roots of GF inoculated garlic plants after 16th day of drought period helps in maintaining high osmotic level in plants suffering from drought stress (30, 32). Similar results are reported in soybean roots during drought period (9). After 16th day of drought period the shoot proline accumulation in non mycorrhizal drought stressed garlic plants was found to decrease. This decrease in accumulation of proline in shoots is an indicator of drought injury (33, 34, 35, 36, 37).

CONCLUSION

Thus, the capacity of AM fungi for increasing plant tolerance to the drought stress imposed may have been related to growth and nutrient uptake improvement. Inoculation of GF was found to be more promising to induce enhancement of antioxidant defense system by acquiring more water and nutrients from soil through extraradical hyphae during drought stress condition. Study also states that the Benefit, WUE analysis help in evaluates the host plant to optimize efficiencies of AM symbiosis under drought stress condition. The strategy of using AM as a biofertilizer will help to improve garlic crop under drought prone areas.

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