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# ARBUSCULAR MYCORRHIZAL FUNGI ENCOURAGE DROUGHT TOLERANCE OF *Chlorophytum borivilianum* BY ENHANCING ANTIOXIDANT ENZYME SYSTEM

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## ABSTRACT

***Chlorophytum borivilianum,*** **due to the presence of high valued industrially important steroidal saponins in its roots, has attracted pharmacological societies worldwide and motivated the farmers of arid and semi-arid zones to cultivate this medicinal herb on commercial scale. However, drought stress is a major abiotic constraint in the successful cultivation of this crop in these areas. One possible way to enhance its production is to improve its drought tolerance through arbuscular mycorrhizal symbiosis. The mechanism by which mycorrhizal symbiosis protects the plants against reactive oxygen species, induced by drought stress, is investigated by evaluating the activity of a set of antioxidant enzymes in relation to different mycorrhizal inocula at various growth stages. The influence of three mycorrhizal inocula viz., *Glomus fasciculatum, G. intraradices* and *G. mosseae* on superoxide dismutase, catalase, glutathione reductase, glutathione-s-transferase, polyphenol oxidase and peroxidase enzyme activities were evaluated in well-watered and drought stressed conditions. The result revealed that the entire mycorrhizal plant materials showed higher drought tolerance effect than non-mycorrhizal ones by enhancing antioxidant enzyme activities. Antioxidant activities were more at 180 days of crop harvest. Therefore, it is recommended to harvest *C. borivilianum* roots at 180 days crop age. Mycorrhizal drought-stressed roots showed significantly higher anti-oxidant activities than the well-watered conditions. Thus, it can be assumed that higher antioxidant activities in roots of mycorrhizal plants might have contributed to alleviate the oxidative damage to biomolecules. The cultivation of *C. borivilianum* with arbuscular mycorrhizae in arid-zone, therefore, can be a value addition against drought stress.**

**Keywords:** Antioxidant enzymes, arbuscular mycorrhiza, *Chlorophytum borivilianum,* drought stress, *Glomus* species

## INTRODUCTION

*Chlorophytum borivilianum* Santapau & R.R. Fern., commonly known as 'Safed musli', is an endangered medicinal herb valued for its dried, fasciculated storage roots which possess immunomodulatory, anti-stress and aphrodisiac properties that forms an important ingredient of herbal tonics to cure impotency, sterility and enhance male potency (Puri, 2003). Steroidal saponins are the main active principle components of roots used as stimulants and metabolic enhancers and reportedly possess anti-tumour activity (Mimaki *et al.*, 1996). In India, *C. borivilianum* is mainly distributed in southern Rajasthan, north Gujarat and western Madhya Pradesh (Maiti and Geetha, 2005). Due to its high pharmaceutical value it has been included in the list of highly traded medicinal plants of India.

Arbuscular mycorrhizae (AM) are widespread under natural conditions and colonize the roots of almost all vascular plants and develop a complex system of extra-radical hyphae under arid conditions (Requena *et al.,* 2001). This helps the transport of immobile nutrients, especially phosphorus, to the plant (Toro *et al.,* 1997). AMF form extensive hyphal networks in soil and provide nutrients to the plants in lieu of assimilates (Smith and Read, 1997). AMF can act as support systems for seedling establishment (Vander Heijden, 2004) and influence the plant invasion success (Klironomos, 2002; Stampe and Daehler, 2003).

Drought stress is considered to be one of the most important abiotic factor limiting the growth and development of semi-arid plants (Kramer and Boyer, 1997). Also, recurrent dry periods and erratic rainfall patterns have determined water shortage and consequent loss or damage in crop production generally in well-rain-fed areas (Vallino *et al.,* 2009). Drought-stress damage the cells directly or indirectly through the formation of activated oxygen species (AOS) such as superoxide radicals and $H_2O_2$ (Mittler, 2002). The detrimental effect of reactive oxygen species (ROS) including $H_2O_2$ is combated by defence mechanisms in plant system involving enzymatic and non-enzymatic antioxidant system. Fortunately, it has been demonstrated that AM symbiosis can protect host plants against the detrimental effects of water deficiency (Auge, 2001; Ruiz-Lozano *et al.,* 2008). Several studies suggest numerous mechanisms by which AM symbiosis can alleviate drought stress in host plants. The most important ones include: direct uptake and transfer of water through fungal hyphae to the host plant (Hardie, 1985), enhancement of plant gas exchange and water-use efficiency (Auge *et al.,* 1992), and protection against the oxidative damage generated by drought (Ruiz-Lozano *et al.,* 2001; Porcel *et al.,* 2003). The improvement of stress tolerance is often related to the enhancement of contents of antioxidant compounds in plants.

In plants, metabolism of reactive oxygen species (ROS) such as superoxide radicals ($O^{2-}$), hydrogen peroxide ($H_2O_2$) and hydroxyl radicals ($^{\cdot}OH$) are kept in dynamic balance. Under water stress conditions antioxidant systems are needed to decrease the damage of tissues (Foyer *et al.,* 1994). Major antioxidant enzyme systems include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), polyphenol oxidase (PPO) and glutathione reductase (GR) (Foyer *et al.,* 1994). Enzyme SOD converts $O^{2-}$ to $H_2O_2$. CAT eliminates $H_2O_2$ by breaking it down to $H_2O$ and $O_2$. GR reduces the oxidized glutathione (GSSG) by using NADPH. Glutathion-s-transferases (GST) are a collection of multi-functional proteins commonly found in all organisms. Plant GST's catalyzes reduction of $H_2O_2$ to less harmful alcohol (Roxas *et al.,* 2000).

With the growing importance of natural drugs and its demand in traditional health care system, overexploitation of *C. borivilianum* in its natural habitat makes the species threatened and hence its cultivation is practiced. The objective of this work was to investigate whether AM symbiosis can help *C. borivilianum* to overcome the negative effects of drought stress by enhancing antioxidant system of this species and to optimize the harvesting period in response to their different active antioxidant enzymes in such adverse condition. The influence of AM fungal inoculation on antioxidant enzyme activities like SOD, CAT, PRO, PPO, GR, and GST in roots of *C. borivilianum* at different harvesting periods under drought conditions were studied.

## MATERIALS AND METHODS

### *Soil and biological materials*

The soil was collected from barren lands of Jodhpur (Rajasthan) under arid environment, sieved (2 mm), diluted with quartz-sand (<1 mm) [1:1, soil: sand, v/v] and sterilized by steaming at 100°C for 1 h for 3 consecutive days. Earthen pots (50×30 cm) of 25 kg capacity were filled with 20 kg soil. Healthy tubers of *Chlorophytum borivilianum* were purchased from the Rajasthan Seed Corporation, Indore, India. One tuber weighing 8-10 g was sown in each pot. Mycorrhizal inoculum was bulked in open-pot culture of *Zea mays* L. and consisted of soil, spores, mycelia and infected root fragments. The inoculum was added @ 10 g $pot^{-1}$ at the sowing time just below the planting tubers.

***Symbiotic development***

The experiment was conducted in a randomized complete block design. The treatments comprized of four plant inoculations viz., *Glomus fasciculatum* ($M_1$), *G. intraradices* ($M_2$) and *G. mosseae* ($M_3$) and non-mycorrhizal plant material (C) evaluated under two conditions viz., well-watered (CW, $M_1W$, $M_2W$ and $M_3W$) and drought-stressed (CD, $M_1D$, $M_2D$ and $M_3D$) conditions. Plants in well-watered conditions were maintained at 100% water holding capacity (WHC) whereas the plants in drought-stressed conditions were maintained at 30% WHC. The watering was done on every alternate day to maintain the desired WHC. An inoculum load of 15,000 spores $pot^{-1}$ (750 spores $kg^{-1}$ soil) was placed in each AM treatment. Eight replicates for each treatment were maintained. Plants were grown under controlled green-house conditions with 80% RH, day/night temperature of 25/15°C, and a photo-period of 16 h at a photosynthetic photon flux density (PPFD) of 460-500 µmol $m^{-2}s^{-1}$. No manure or fertilizer was applied at any stage of plant growth. Eight pots of each watered and drought stressed plants were harvested at 45, 90, 180 and 270 days of growth stages.

To assess the root colonization by VAM fungi, standard staining technique of Philips and Hayman (1970) was followed. The plant roots were cleared with 10% (w/v) KOH at 90°C for 12-15 min. in a water bath, rinsed three times, stained in 0.1% trypan blue (made in lactophenol) at 90°C for 3–5 min. and mounted in lactic acid : glycerol (1:1). Spores of different species were extracted by wet sieving and decantation technique of Gerdemann and Nicolson (1963). Total number of mycorrhizal fungal spores in soil samples was estimated by the method of Gaur and Adholeya (1994) and all the spores were examined using Medilus-20 TR compound microscope.

***Soil and plant analysis***

Soil analysis (EC, organic C, total N, P fractions and K content) was done as described by Jackson (1967). Root samples (0.5 g fresh mass) were homogenized in 50 mM Na-phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinyl pyrrolidone. The homogenate was filtered through four layers of cheese-cloth and then centrifuged at 4ºC for 20 min. at 15,000 g. All operations of enzyme extraction were performed at 0-4ºC and enzyme assays carried out at room temperature (25±1ºC).

***Antioxidant enzyme assays***

Total superoxide dismutase (SOD) activity was assayed as per the method of Becana *et al.* (1986) following the inhibition of photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture contained 50 mM Na-phosphate (pH 7.8), 0.1 mM EDTA, 14.3 mM methionine, 82.5 lM NBT and 2.2 lM riboflavin. The reaction was initiated by placing the test tubes under 15 W fluorescent lamps. The reaction was terminated after 10 min. by removing the reaction tubes from the light source. Non-illuminated and illuminated reactions without supernatant served as calibration standards. The reaction products were measured at 560 nm. One unit of SOD (U) was defined as the amount of enzyme that produced a 50% inhibition of NBT reduction under assay conditions.

Catalase (CAT) activity was measured by following the decomposition of $H_2O_2$ at 240 nm in a reaction mixture containing 50 mM phosphate buffer (pH 7.0), 15 mM $H_2O_2$ as described by Chance and Maehly (1955) for 2 min. Enzyme activity was expressed as l mol ascorbate oxidized $min^{-1}$ $mg^{-1}$ protein. Glutathione reductase (GR) activity was measured according to Shaedle and Bassham (1977). The decrease in absorbance at 340 nm was followed in a reaction mixture containing 50 mM tris-HCl buffer (pH 7.5), 0.5 mM GSSG, 0.1 mM EDTA, 3 mM $MgCl_2$ and 0.15 mM NADPH. Specific activity was expressed as mol NADPH oxidized $min^{-1}$ $mg^{-1}$ protein. Glutathione-s-transferase (GST) activity was assayed as reported by Mannervik and Guthenberg (1981) by following the change in absorbance at 340 nm for 3 min. in a reaction mixture containing 100 mM Na-phosphate buffer (pH 6.5), 1 mM GSH and 1-chloro-2,4 dinitrobenzene. The activity of GST was expressed as l mol dinitrophenyl glutathione formed $min^{-1}$ $mg^{-1}$ protein.

Polyphenol oxidase (PPO) and peroxidase (PRO) activities were measured spectrophotometrically using l-3,4-dihydroxyphenylalanine (DOPA) as the substrate (Sinsabaugh *et al.*, 2003). For

phenol oxidase, 50 µl of 25 mM DOPA was added to each sample well. Peroxidase assays received 50 µl of 25 mM DOPA plus 10 µl of 0.3% $H_2O_2$. Negative control wells for phenol oxidase contained 200 µl acetate buffer and 50 µl DOPA solution; blank wells contained 200 µl sample suspension and 50 µl acetate buffer. For peroxidase, negative control and blank wells also contained 10 µl $H_2O_2$. There were 16 replicate sample wells for each assay and 8 replicate wells for blanks and controls. The microplates were incubated in dark at 20°C, approximately 4 h for peroxidase and 18 h for phenol oxidase. Activity was quantified by measuring absorbance at 450 nm using a microplate spectrophotometer and expressed as µm $min^{-1}$ $mg^{-1}$ protein.

***Statistical analysis***

The data was analyzed using one-way ANOVA, followed by Turkey multiple range test with level of significance set at $P_{0.05}$. Statistical calculations were performed using SPSS version 15.0 statistical package.

## RESULTS

The physico-chemical characteristics of soil revealed that was soil loamy sandy type having 22.3% WHC and poor in phosphorus and potassium (Table 1).

***Inoculum establishment***

Arbuscular mycorrhizae with arbuscules, which are the structural and functional criterion of symbiosis, were observed in case of all inoculated plant materials after two weeks. No other root endophyte was detected in the investigated materials. Root infection was more in mycorrhizal plant materials than in non-mycorrrhizal samples both under well-watered and drought conditions. Average root infection and spore build up was significantly higher in mycorrhizal plants (Table 2). However, the root infection of *C. borivilianum* showed no significant changes within the drought and well-watered conditions although slightly more infection and spore build up was observed under drought condition. In general, more root infection and spore build up was observed under *Glomus intraradices* inoculation.

**Table 1: Physico-chemical characteristics of the soil used in the experiment (each value is the mean of five soil samples)**

| | |
|---|---|
| Soil texture | Loamy sandy |
| - Sand (%) | 74.8 |
| - Silt (%) | 15.2 |
| - Clay (%) | 10.0 |
| WHC (%) | 22.3 |
| pH ($H_2O$) | 7.90 |
| Electrical conductivity ($dSm^{-1}$) | 0.13 |
| Total organic carbon (%) | 0.03 |
| Total N (mg $kg^{-1}$) | 280.92 |
| Olsen's P (mg $kg^{-1}$) | 3.58 |
| Organic P (mg $kg^{-1}$) | 60.15 |
| Total P (mg $kg^{-1}$) | 187.84 |
| Potassium (mg $kg^{-1}$) | 25.25 |

***Effect of AM on antioxidant enzyme activities***

Arbuscular mycorrhizal inoculation helped to increase the antioxidant enzyme activities in the roots of *C. borivilianum*. However, the effect of mycorrhizal inoculation treatment level depended on the compatibility

**Table 2: Average root infection and spore number at 45 days of crop age**

| Treatment | Well watered condition | | Drought condition | | LSD* (0.05) |
|---|---|---|---|---|---|
| | Root infection (%) | Spore number (100 $g^{-1}$ soil) | Root infection (%) | Spore number (100 $g^{-1}$ soil) | |
| Control | 40.3 | 41.0 | 42.9 | 46.0 | 3.92 |
| *Glomus fasciculatum* | 75.8 | 78.7 | 77.6 | 83.5 | 5.05 |
| *G. intraradices* | 92.3 | 152.5 | 93.0 | 156.5 | 9.12 |
| *G. mosseae* | 79.9 | 123.5 | 81.1 | 128.0 | 7.23 |
| $CD_{0.05}$ | 4.10 | 10.23 | 3.92 | 11.35 | |

*Comparison of spore number between well watered and drought condition

of root, mycorrhizal species used and the conditions of experimental soil. Activities of all the antioxidant enzymes were significantly higher in mycorrhizal infected plant materials than in non-mycorrhizal samples. Also, it was observed that drought-stress enhanced antioxidant activities in all the AM colonized materials including control (CD). The CAT and SOD activities were significantly higher in mycorrhizal well-watered conditions ($M_1W$, $M_2W$ and $M_3W$) than the non-mycorrhizal environment (CW). Mycorrhizal drought stressed roots ($M_1D$, $M_2D$ and $M_3D$) exhibited very high CAT and SOD activities than those under well-watered conditions (Fig. 1 and 2). However, $M_2D$ and $M_3D$ did not show any significant response with respect to CAT and SOD activities. Also, the mycorrhiza-colonized plants showed strong induction of CAT and SOD activities at 180 days of harvesting in both drought and well-watered conditions (Fig. 1 and 2). Both the activities, however, decreased after 90 days of harvesting in case of $M_1D$.

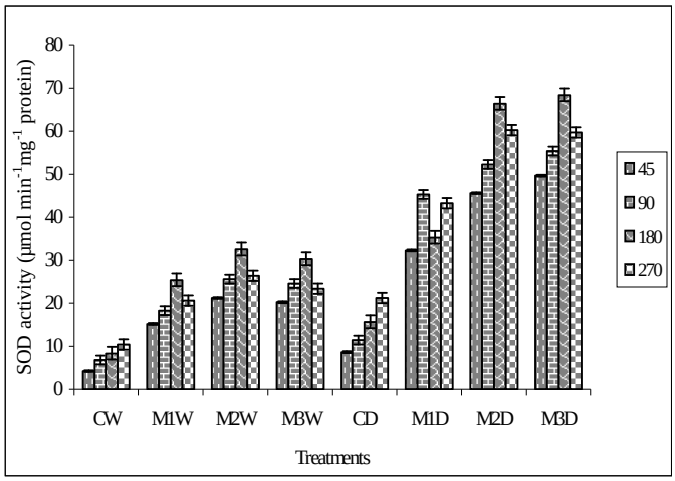


**Fig. 1: Effect of AM fungi on superoxide dismutase (SOD) activities in roots of *C. borivilianum* under well-watered (W) and drought-stress (D) conditions at different harvesting periods. Bar represents mean ±SE of ten independent replicates at p <0.05 determined by Turkey multiple range test. $M_1$ - *Glomus fasciculatum*, $M_2$ - *G. intraradices*, $M_3$ - *G. mosseae* and CD - Control drought-stressed**

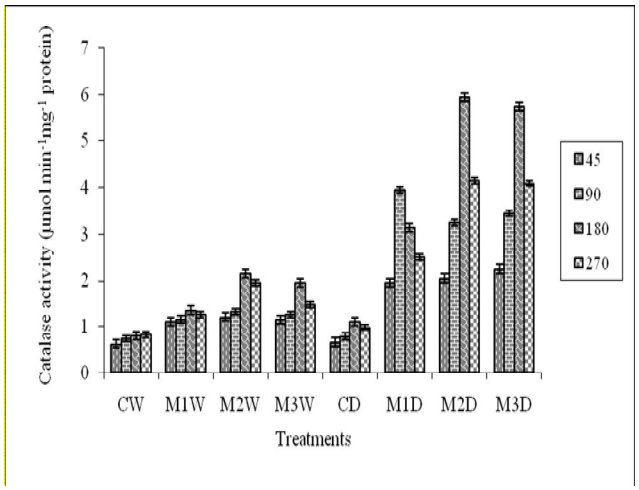


**Fig. 2: Effect of AM fungi on catalase (CAT) activities in roots of *C. borivilianum* under well-watered (W) and drought-stress (D) conditions at different harvesting periods. Bar represents mean±SE of ten independent replicates at p <0.05 determined by Turkey multiple range test. $M_1$ - *Glomus fasciculatum*, $M_2$ - *G. intraradices*, $M_3$ - *G. mosseae* and CD - Control drought-stressed.**

Mycorrhizal infected roots of *C. borivilianum* showed significantly higher GR and GST activities than the non-mycorrhizal materials (CW and CD). The GR activity was optimum at 180 days of harvesting period in all the drought- conditioned roots colonized by arbuscular mycorrhizae. $M_2D$ and $M_3D$ infected materials were more effective in inducing GR activities under drought-stress condition than under well-watered situation (Fig. 3). But, no significant activities were found within these two mycorrhizal roots. $M_1D$ and $M_3D$ were exposed a constant increase in GST activities with the increase in harvesting periods in drought stress condition (Fig. 4). GR and GST activities were very low in critical growth stage (45 d).

Mycorrhizal infected roots of *C. borivilianum* showed significantly higher GR and GST activities than the non-mycorrhizal materials (CW and CD). The GR activity was optimum at 180 d of harvesting period in all the drought-conditioned roots colonized by arbuscular mycorrhizae. $M_2D$

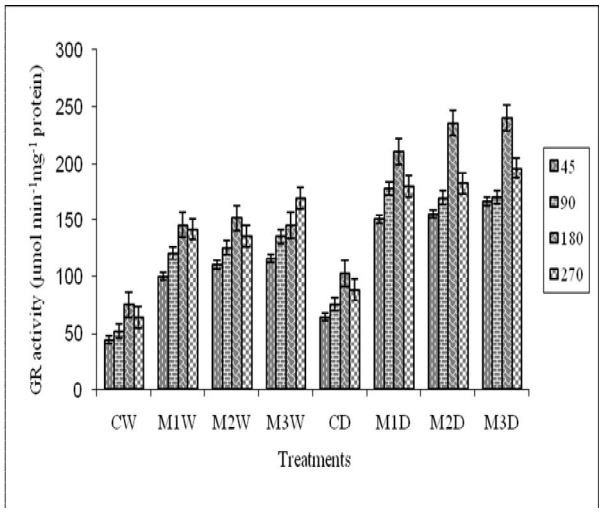


**Fig. 3: Effect of AM fungi on glutathione reductase (GR) activities in roots of *C. borivilianum* under well-watered (W) and drought-stress (D) conditions at different harvesting periods. Bar represents mean±SE of ten independent replicates at p <0.05 determined by Turkey multiple range test. $M_1$ - *Glomus fasciculatum*, $M_2$ - *G. intraradices*, $M_3$ - *G. mosseae* and CD - Control drought-stressed.**

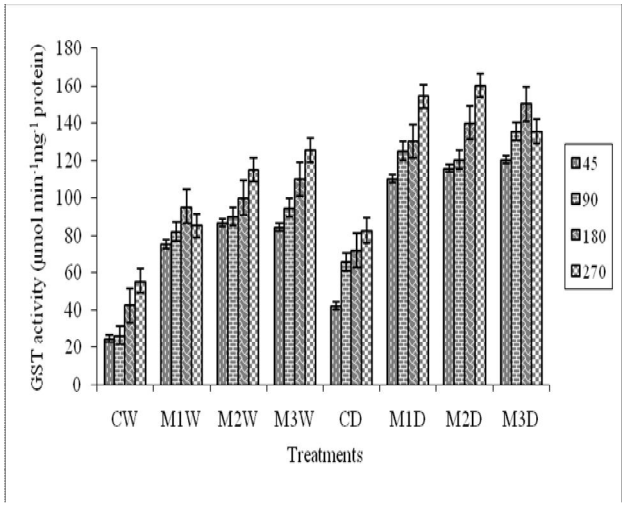


**Fig. 4: Effect of AM fungi on glutathione-s-transferase (GST) activities in roots of *C. borivilianum* under well-watered (W) and drought-stress (D) conditions at different harvesting periods. Bar represents mean±SE of ten independent replicates at p <0.05 determined by Turkey multiple range test. $M_1$ - *Glomus fasciculatum*, $M_2$ - *G. intraradices*, $M_3$ - *G. mosseae* and CD - Control drought-stressed.**

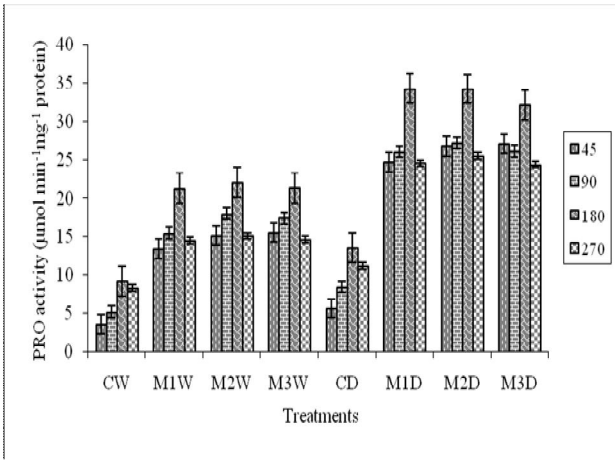


**Fig. 5: Effect of AM fungi on polyphenol oxidase (PPO) activities in roots of *C. borivilianum* under well-watered (W) and drought-stress (D) conditions at different harvesting periods. Bar represents mean ±SE of ten independent replicates at p <0.05 determined by Turkey multiple range test. $M_1$ - *Glomus fasciculatum*, $M_2$ - *G. intraradices*, $M_3$ - *G. mosseae* and CD - Control drought-stressed.**

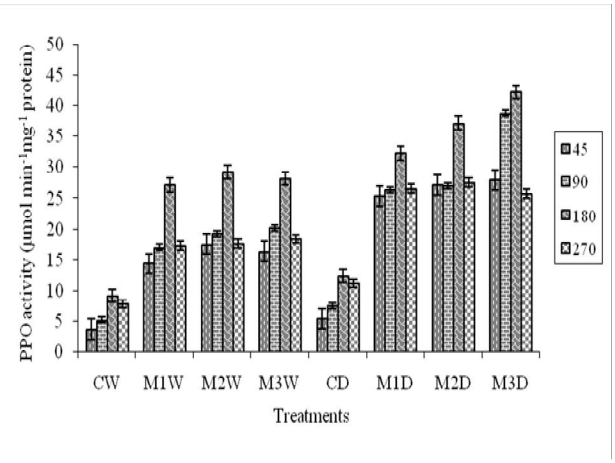


**Fig. 6: Effect of AM fungi on peroxidase (PRO) activities in roots of *C. borivilianum* under well-watered (W) and drought-stress (D) conditions at different harvesting periods. Bar represents mean±SE of ten independent replicates at p <0.05 determined by Turkey multiple range test. $M_1$ - *Glomus fasciculatum*, $M_2$ - *G. intraradices*, $M_3$ - *G. mosseae* and CD - Control drought-stressed.**

and $M_3$D infected materials were more effective in inducing GR activities under drought-stress condition than under well-watered situation (Fig. 3). No significant activities were found within these

two mycorrhizal roots. $M_1D$ and $M_3D$ showed constant increase in GST activities with increase in harvest period in drought-stress condition (Fig. 4). GR and GST activities were very low in critical growth stage (45 d).

There was marked differences in PRO and PPO activities between inoculated roots under drought-stress and well-watered conditions. A significant difference was also observed between different types of mycorrhizal treatments. However, $M_1D$ and $M_2D$ exhibited very high PRO activities followed by $M_3D$ at 180 d of harvesting period (Fig. 5). Also, $M_3D$ was responsible for optimum PPO activity in drought-stress at 180 d of growth stage followed by 90 d (Fig. 6). Drought stress proved to be the major significant factor in inducing PRO and PPO activities in all the mycorrhizal inoculated plant materials. In general, *C. borivilianum* roots possessed more antioxidant enzyme system at 180 d of growth stage.

## DISCUSSION

Mycorrhizal symbiosis causes important changes in plant metabolism and growth (Arines *et al.,* 1994), although very little is known about the biochemical mechanism involved in this association. AM symbiosis is formed by approximately 80% of vascular plants (Smith *et al.,* 2010). Low soil water content is strongly linked to low nutrient availability and poor soil structure, therefore mycorrhizal development plays significant role in the alleviation of both water and nutrient deficiency. In a similar way, low nutrient soils in our case (mostly P deficit soil) supported the plants that lived symbiotically with the organisms that enhance nutrient acquisition (Fitter, 2005). Mycorrhizal inoculation was very effective in increasing antioxidant enzyme activities which is in agreement with Ruiz-Lozano *et al.* (1996) who found that *Lactuca sativa* plants colonized with *G. deserticola* showed the development and stimulation of defense system. Mycorrhizal inoculation causes biochemical perturbations in plants thereby leading to the changes in antioxidant defense system (Beak and Skinner, 2003). In arid area drought stress is most common abiotic factor affecting plant cultivation. Mycorrhizal fungi are known to enhance water absorption by plants grown under water-deficit condition owing to altered root morphology or contribution by soil hyphae (Auge, 2001).

In present study, roots exhibited an increase in the activities of $H_2O_2$ scavenging enzymes like catalase (CAT) and superoxide dismutase (SOD) in all the plant materials infected by AM. Fortunately, plants have developed various protective mechanisms to eliminate or reduce ROS, which are effective at different levels of stress-induced deterioration (Beak and Skinner, 2003). The enzymatic antioxidant system, found in various cell compartments, is one of the protective mechanisms including SOD and CAT, help in catalysing the disproportion of two $O^{2-}$ radicals to $H_2O_2$ and $O_2$ (Scandalios, 1993). Moreover, ROS are inevitable byproducts of cell metabolism (Martinz *et al.,* 2001). But under normal (well-watered) conditions the production and destruction of ROS is well-regulated in cell metabolism (Mittler, 2002). When a plant faces harsh conditions, ROS production overcomes the scavenging systems and oxidative stress bursts. In these conditions, ROS attacks vital biomolecules and disturbs cell metabolism which ultimately leads to the cell death (Sakihama *et al.,* 2002). SOD plays a prime role in protecting cells against oxidative stress. However, dismutation of $O^{2-}$ radicals simply serves to convert one destructive ROS to another. Since $H_2O_2$ is a strong oxidant that rapidly oxidizes thiol groups, it cannot be allowed to accumulate in excess (Noctor and Foyer, 1998). In our study SOD activities were high when exposed to drought-stress and inoculated by AM. Reports have revealed that SOD activity was higher in shoots and roots of *Lactuca sativa* colonized by *G. mosseae* than in non-AM samples under water-stressed conditions (Ruiz-Lozano *et al.,* 1996). AM symbiosis increased the expression of the Mn-SODII gene in water-stressed lettuce plants, and this correlated well with plant tolerance to water stress (Ruiz-Lozano *et al.,* 2001). Similarly in our case AM may probably have induced new SOD isoenzymes in mycorrhizal roots. The response of AM colonization to SOD warrants more extensive study in future.

In all living organisms, glutathione (GSH) is the major low molecular weight thiol-containing compound, present in millimolar concentrations in different tissues where it serves as a general reductant (Foyer *et al.,* 1991). In addition to the induction of enzymes, it helps in the removal of toxic oxygen derivatives in ascorbate-glutathione cycle and participates in sulphur metabolisms and gene expression (Foyer *et al.,* 1995). GR is a key enzyme in ascorbate-glutathione pathway that maintains higher GSH/GSSG ratio. A higher GR activity in the roots of *C. borivilianum* demonstrates its superior tolerance mechanism in maintaining redox state. Although it has been suggested that enhanced GSH content is not in itself sufficient to improve resistance to high rates of ROS production (Noctor and Foyer, 1998), the increase in GR activity (the enzyme which regenerates the oxidized GSH into its reduced form) observed in mycorrhizal plants presumably helps in alleviating the damage caused by drought stress. The results suggest that consistently higher GR and GST activities in the roots of mycorrhizal plants might have generated reduced antioxidants (GSH), which helped to decrease oxidative damage in order to sustain roots for longer time. Also, the activities were optimum at 180 d of growth stage. Polyphenol oxidases (PPOs) catalyze the $O^{2-}$ dependent oxidation of mono- and *o*-diphenols to *o*-diquinones, highly reactive intermediates, the secondary reactions of which are believed to be responsible for the oxidative browning that occurs as a consequence of plant senescence, wounding and pathogen infection (Abo-Ghalia and Khalafallah, 2008). Also, in present study the oxidative damage in drought-stressed mycorrhizal plant materials might have helped to induce the PPO response. It is reported that peroxidases are responsible for cell wall lignifications and other cell wall stiffening processes which conclude in the maturation of cell wall (Prasadi *et al.,* 2004). The concentration of two enzymes was greater at heading stage of plant growth, which may be due to the increased effect of drought stress at critical stage of growth. Previously Wu *et al.* (2006a,b, 2008), Auge *et al.* (2007*)* and Roldan *et al.* (2008) have observed similar pattern of antioxidant enzyme activities by AM. Under drought conditions *G. mosseae and G. intraradices* colonization stimulated PPO and PRO activities against oxidative damage. However, on harvesting of roots after 180 d all the mycorrhizal inoculated plants significantly induced higher scavenging activities than non-mycorrhizal material.

Drought causes an oxidative stress in plants and many of the degenerative reactions associated with abiotic stress are mediated by reduced oxygen. In relation to the effect of different AM used, it is clear that the symbiotic efficiency of *G. intraradices and G. mosseae* was higher than that of *G. fasciculatum*. The diverse behaviour of three *Glomus* species used in our study could be related to their varying developmental patterns. Normally, *G. intraradices* is a very aggressive AM fungus in terms of intensity of root colonization and production of fungal structures such as vesicles and chlamydospores (Graham *et al.,* 1996).

AM fungi help plants in enhancing their nutrients uptake and drought tolerance efficiency by inducing defence enzymes subjected to water-stress (arid soil) at critical stages of plant growth followed by recovery. The present study suggested that in order to increase crop yield arbuscular mycorrhizae may be used extensively especially for the crops that are often cultivated in the farms which are often subject to water-stress at critical growth stages. Since *Chlorophytum borivilianum* is a crop of industrial importance and highly traded medicinal plant, mycorrhizal inoculation can be used as a better alternative against chemical fertilizers and farmyard manure which is a costly affair for its large scale production.

**Conclusion:** The present study suggested that arbuscular mycorrhizal fungal colonization helps in the enhancement of enzymatic antioxidant production which, in turn, helps AM plants in drought tolerance. Also, AM can be used as an effective source for increasing plant production values in arid and semi-arid areas. Cultivation of *C. borivilianum* with AM inocula can help the species to improve nutrient acquisition as well as to alleviate oxidative damage in order to sustain the species for better yield under drought stress conditions.

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