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GENE NOTE

# Cloning of cDNAs encoding SODs from lettuce plants which show differential regulation by arbuscular mycorrhizal symbiosis and by drought stress

Juan M. Ruiz-Lozano[1], Carlos Collados, José M. Barea and Rosario Azcón

Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín (CSIC), Profesor Albareda No. 1, E-18008 Granada, Spain

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**Abstract**

In the present study three cDNA fragments were cloned using degenerate primers for *Mn-sod* genes and PCR: two showed a high degree of identity with *Mn-sod*s from plants and the third with *Fe-sod*. Arbuscular mycorrhizal (AM) symbiosis down-regulated their expression pattern under well-watered conditions. In contrast, AM symbiosis in combination with drought stress considerably increased the expression of the *Mn-sod II* gene and this correlated well with plant tolerance to drought. These results would suggest that mycorrhizal protection against oxidative stress caused by drought may be an important mechanism by which AM fungi protect the host plant against drought.

Key words: Arbuscular mycorrhiza, drought stress, oxidative stress, SOD.

In plants, the role of SOD during environmental adversity has received much attention as superoxide radicals have been found to be produced under various types of stress conditions (Bowler *et al*., 1992). In a previous study it was found that mycorrhizal plants increased SOD activity in both shoot and root tissues as a consequence of drought, while P-fertilized uninoculated plants did not increase such activity under drought conditions. This increase of SOD activity in AM plants was directly correlated with enhanced plant production and drought resistance (Ruiz-Lozano *et al*., 1996).

To date, however, there is little information on SODs in AM symbiosis, mainly at the molecular level. A CuZn-SOD isoform has been reported in spores of *Glomus mosseae* (Palma *et al*., 1993). These authors also found that mycorrhizal red clover roots had two new SOD isozymes: a mycCuZn-SOD and a Mn-SOD II. The mycCuZn-SOD was found to be specific for the mycorrhizal association, whereas the Mn-SOD II was also present in red clover nodules. The authors suggested that the Mn-SOD II could be induced in mycorrhizal roots by the presence of the fungus. The objective of this work was the cloning of *Mn-sod* genes in lettuce plants and the analysis of their expression pattern in mycorrhizal roots subjected or not to drought stress.

Seeds of *Lactuca sativa* L. cv. Romana were inoculated with one of two AM species as described previously (Ruiz-Lozano *et al*., 1996). The AM fungi were *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe, isolate BEG 122 and *Glomus intraradices* (Schenck and Smith) isolate BEG 121. Uninoculated P-fertilized plants were also obtained. Plant growth conditions and measurements were as described previously (Ruiz-Lozano *et al*., 1996) and the parameters of mycorrhizal colonization were determined (Trouvelot *et al*., 1986). Drought decreased plant biomass production by 30% in P-fertilized plants and by 10–16% in *G. intraradices*- and *G. mosseae*-colonized plants, respectively (Table 1). The frequency of colonization (*F*) was similar for both fungi and was unaffected by drought stress. The intensity of root colonization (*M*) and the arbuscule abundance (*A*) were higher in *G. intraradices*-colonized plants than in those colonized by *G. mosseae*. These two parameters were not affected by drought stress.

Total RNA (2.5 μg) from mycorrhizal roots (*G. mosseae*) subjected to drought stress was reverse transcribed to cDNA using AMV-RT enzyme and oligo(dT)$_{15}$ primer, in a final volume of 25 μl. A cDNA fragment of about 440 bp was amplified by PCR and degenerate primers designed for *Mn-sod* genes. The sequence of primers were 5′-AC(C or A)(A or C)GAA(G or A)CACCA(C or T)CA(G or A)ACTTA-3′ for the forward primer and 5′-TG(C or G)A(A or G)GTAGTAGGCATG(T or C)TCCCA-3′ for the reverse primer. The amplified cDNA was cloned into the pGME plasmid (Promega). DNA from several independent clones was isolated for sequencing. Similarity searches were carried out in the EMBL databank, using the BLAST software. A cDNA fragment (*Mn-sod I*, Accession No. AJ310449) showed the highest similarity (80% identity) with the *Mn-sod* gene from *Hevea brasilensis*. A second cDNA fragment (*Mn-sod II*, Accession No. AJ310448) showed the highest similarity (78% identity) with the *Mn-sod* gene from *Ipomoea batatas*. Finally, a cDNA fragment of 474 bp (Accession No. AJ310450) showed the highest similarity (73% identity) with the *Fe-sod* gene from *Vigna unguiculata*. The identity between both *Mn-sod* sequences was 87%, while that between *Mn-sod I* and *Fe-sod* was 57% and the identity between *Mn-sod II* and *Fe-sod* was 56%. The plant origin of these three cDNA fragments was established by PCR on genomic DNA using sequence specific primers for each cDNA (data not shown).

Northern blotting was performed as described previously (Ruiz-Lozano *et al*., 1999) on total RNA from non-mycorrhizal and mycorrhizal roots of lettuce plants subjected or not to drought stress and the data were normalized according to the amount of rRNA in the membranes. Both *Mn-sod* genes showed different expression patterns with regard to mycorrhizal symbiosis and drought stress

[1] To whom correspondence should be addressed. Fax: +34 958 129600. E-mail: jmruiz@eez.csic.es

**Table 1.** *Shoot dry weight (g) and fungal colonization in P-fertilized or mycorrhizal (*G. mosseae*, Gm or* G. intraradices*, Gi) lettuce plants cultivated under well-watered (Ww) or drought-stressed (D) conditions*

*F* (colonization frequency), *M* (colonization intensity), *A* (arbuscule abundance in the whole root system).

| Treatment | Shoot dry weight | Mycorrhizal infection | | |
|---|---|---|---|---|
| | | *F* (%) | *M* (%) | *A* (%) |
| P-fertilized Ww | 1.25 ab | 0 b | 0 c | 0 d |
| P-fertilized D | 0.88 d | 0 b | 0 c | 0 d |
| Gm Ww | 1.28 ab | 91 a | 54 b | 40 bc |
| Gm D | 1.07 c | 88 a | 42 b | 29 c |
| Gi Ww | 1.35 a | 91 a | 68 a | 53 ab |
| Gi D | 1.18 bc | 91 a | 78 a | 68 a |

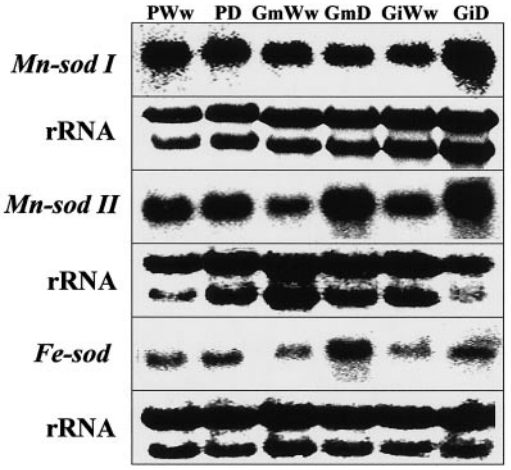


**Fig. 1.** Northern blot of root total RNA (20 μg) from P-fertilized (P) or mycorrhizal (*G. mosseae*, Gm or *G. intraradices*, Gi) lettuce plants cultivated under well-watered (Ww) or drought-stressed (D) conditions. Blots were hybridized with probes for *Mn-sod I*, *Mn-sod II* or *Fe-sod* genes. The panel under each Northern analysis shows the amount of rRNA loaded for each treatment (methylene blue staining). Northern blot analyses were repeated twice using two different sets of plants and RNA for all treatments.

(Fig. 1). *Mn-sod I* showed a slight decrease (ranging from 2% to a maximum of 17%) of gene expression in mycorrhizal roots. A more pronounced decrease was also observed in *Mn-sod II* gene expression as a consequence of mycorrhiza formation (52% in the case of *G. mosseae* and 29% in the case of *G. intraradices*, relative to non-mycorrhizal plants), but only in well-watered plants. These results do not agree with previous findings which reported an increase in total SOD activity as a consequence of mycorrhiza formation (Arines *et al*., 1994; Ruiz-Lozano *et al*., 1996). However, in this work, only the expression of *Mn-sod* genes of plant origin was being determined, while total activity, including that of CuZn-SOD, was measured in other studies. Hence, it is likely that CuZn-SOD, which can be present in both the plant and the AM fungus (Palma *et al*., 1993), is responsible for the increased total SOD activity previously described (Arines *et al*., 1994; Ruiz-Lozano *et al*., 1996) in mycorrhizal plants. Supporting these present results, Martín *et al*. recently found no increase in total SOD activity in well-watered mycorrhizal onion plants of different ages, as compared to uninoculated plants (Martín *et al*., 1998).

In contrast, under drought conditions, *Mn-sod II* considerably increased its level of transcript accumulation in mycorrhizal (over 50% in *G. mosseae*-colonized plants and over 138% in *G. intraradices*-colonized plants relative to non-mycorrhizal ones). This increase in gene expression correlated with enhanced tolerance to drought, in terms of plant growth maintenance, by both mycorrhizal treatments. Transgenic alfalfa plants overexpressing Mn-SOD were found to have increased tolerance to herbicides, freezing stress and water deficit (McKersie *et al*., 1993, 1996).

The cloning of a *Fe-sod* gene when using degenerate primers designed for Mn-SODs can be explained by the high structural similarity between Mn-SODs and Fe-SODs (Bowler *et al*., 1994). The level of expression of the lettuce *Fe-sod* gene was distinctly lower than those of both *Mn-sod* genes. Fractionation studies have indicated that, when Fe-SOD is present, it is located in the plastids (Bowler *et al*., 1994). It is, therefore, thought that the cloned cDNA fragment encoding a putative Fe-SOD in lettuce roots must also come from the root plastids. Whether or not there is a role for this Fe-SOD in AM symbiosis or during drought stress has yet to be elucidated since it displayed a different pattern when comparing *G. mosseae*- and *G. intraradices*-colonized roots and, therefore, no clear conclusion can be drawn.

In conclusion, it has been shown that mycorrhizal symbiosis down-regulated the expression pattern of *sod* genes under well-watered conditions. In contrast, mycorrhizal symbiosis in combination with drought stress considerably increased the expression of the *Mn-sod II* gene and this correlated with plant tolerance to drought. These results would suggest that mycorrhizal protection against oxidative stress caused by drought may be an important mechanism by which AM fungi protect the host plant against drought.

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