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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (8): 1326-1330, 2007
ISSN 1028-8880

Effect of Arbuscular Mycorrhiza (*Glomus etunicatum*) on Some Physiological Growth Parameters of Tomato Plant under Copper Toxicity in Solution

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Abstract: In this study, the effect of the Arbuscular Mycorrhizal fungus *Glomus etunicatum* on the physiological growth parameters of tomato (*Lycopersycium esculentum* Mill) plants on the toxicity level of copper was investigated. To explain the physiological growth of these plants, some physiological growth parameters were determined in the shoots and leaves of Arbuscular Mycorrhizal (AM) and non-mycorrhizal (non-AM) plants such as Dry Matter (DM) contents, chlorophyll (chl) content and amount of total sugar. All parameters increased in AM tomato plants compared with those of the non-AM plants. Furthermore, it was determined that P concentration was positively correlated with all chlorophyll and sugar contents. It is concluded that increased P concentration because of the mycorrhizal symbioses, positively affects the physiological performance of tomato plants.

Key words: Arbuscular mycorrhiza, *Glomus etunicatum*, tomato, copper, chlorophyll and sugar

INTRODUCTION

Copper (Cu) is an essential redox-active transition metal that is involved in many physiological processes in plants because it can exist in multiple oxidation states *in vivo*. Under physiological conditions Cu exists as Cu^{2+} and Cu^{+}. Cu acts as a structural element in regulatory proteins and participates in photosynthetic electron transport, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormone signaling (Marschner, 1995; Raven *et al*., 1999).

Toxic levels of Cu occur naturally in some soils whereas others may contain high levels of Cu as a result of the anthropogenic release of heavy metals into the environment through mining, smelting, manufacturing, agricultural and waste disposal technologies. At concentrations above those required for optimal growth Cu was shown to inhibit growth and to interfere with important cellular processes such as photosynthesis and respiration (Marschner, 1995; Prasad and Strzalka, 1999). Plants grown in the presence of high levels of Cu normally show reduced biomass and chlorotic symptoms. A lower content of chlorophyll and alterations of chloroplast structure and thylakoid membrane composition was found in leaves under such growth conditions (Baszynski *et al*., 1988; Lidon and Henriques, 1991; 1993; Ciscato *et al*., 1997; Pätsikkä *et al*., 1998; Quartacci *et al*., 2000).

Arbuscular Mycorrhiza (AM) is one of the most widespread Mycorrhizal associations between soil microorganisms and higher plants. The function of all mycorrhizal systems depends on the ability of the fungal symbiont to absorb inorganic and/or organic nutrients available in soil (Marschner and Dell, 1994). In addition, organic carbon derived from photosynthesis is transferred to these symbionts, which are biotrophic microorganisms and this substance maintains the development of spores and fruit bodies in most mycorrhizae types by translocation of the substance to the growing margins of the extraradical mycelium (Smith and Read, 1997). AM has importance due to its great capability to increase plant growth and yield under certain conditions. The major reason for this increase is the ability of plants in association with AM to uptake some nutrients such as phosphorus efficiently (Podila and Douds, 2001).

As a result of this symbiotic association between AM fungi and host plants, P content also has an effect on physiological parameters in plants (Johnson, 1984; Paradi, 2003). One of the physiological parameters is the increase in photosynthesis.

AMF-colonized plants are generally more resistant to stresses caused by drought, salt, heavy metals or attack by pathogens. These positive effects of the fungi on the growth of plants often result from an improved nutrient supply and can partly be due to complex and not easily resolved interactions between the symbiotic partners.

With regard to heavy metal stress, the literature is somewhat controversial. At low concentrations, several heavy metals such as Zn, Cu, Mn or Mo are micronutrients. Published data indicate that the colonization of roots by AMF results in an enrichment

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of these metal ions in the low concentration range (Dýaz *et al*., 1996; Leyval *et al*., 1997). In contrast, AMF-colonized roots of plants from soils severely polluted by high concentrations of heavy metals show lower amounts of heavy metals than non-colonized plants (Schüepp *et al*., 1987; El- Kherbawy *et al*., 1989; Weissenhorn *et al*., 1995; Kaldorf *et al*., 1999; but see also opposite data by Gildon and Tinker, 1983; Killham and Firestone, 1983). At high heavy metal concentrations, the elements that unavoidably reach the inside of the roots are concentrated in the inner root parenchyma cells, where the intraradical fungal structures carbuscules, vesicles, intraradical hyphae) mainly reside (Kaldorf *et al*., 1999).

MATERIALS AND METHODS

Cultivation of plants: Seeds of tomato (*Lycopersycium esculentum* Mill) were germinated in a moist mix of soil and sand in polystyrene trays. There 28 day old seedlings, uniform in size, were transplanted into 30 plastic pots filled with sterilized sand. Half of the pots received the AMF *Glomus etunicatum* and Trappe by placing 30 g (moist weight) of inoculums is soil below the tomato seedling prior to planting. The AMF inoculum (consist of soil and root fragments and spores) was placed directly adjacent to each seedling root to facilitate fungal colonization of plant roots. Control treatments received no AMF inoculum.

The plants were grown in a greenhouse under natural photoperiods (28/20°C day/night 6000/10000 lux lightin tensity) for 13 weeks during which only distilled water was applied. In addition, twice a week, each pot was supplied with 100 mL of a nutrient solution containing: five Cu concentration (0, 1.5, 3.5, 5.5, 7.5 Mm $CuSO_4$) added to Hogland nutrient solution (with half P concentration).

The symbiotic fungal partner, *Glomus etunicatum*, was produced in a soil:sand (1/1, v/v) mixture using maize as the host plant. Inoculum of *Glomus etunicatum* (30 g), consisting of spores, external mycelium and AMF colonized roots, was laid around the seed. The same amount of sterilized inoculum was laid into the control pots. The percentage of mycorrhizal colonization was estimated by the grid line intersect method (Giovanetti and Mosse, 1980).

At the end of the experiment, plants were harvested 10 weeks after seed sowing. Plant shoots were separated, dried (70°C 48 h) and weighed. The contents of total chlorophyll in leaves were determined by the spectrophotometer according to Smith and Benitez (1955).

The root and shoot length was measured by ruler (Fig. 1 and 2). The content of total chlorophyll in leaves was determined by the spectrophotometer according to Smith and Benitez (1955). Total sugar content in shoots and roots were determined by anthrone method described by Fales (1951).

RESULTS

Figure 1 shows the effect of $CuSo_4$ on the root growth of mycorrhizal and non-mycorrhizal tomato plant. Increasing concentrations of $CuSO_4$ from 0 to 7.5 mM decreased root length, progressively however the length of AM-plants higher than non-AM plant. However, no reduction of shoot length by $CuSO_4$ was observed in Cu treatment and AM and non-AM plants (Fig. 2). The differential effect of Cu on root and shoot growth could be accounted by the fact that Cd is accumulated mainly in roots and to a minor extent in shoots (Parviz Malekzadeh *et al*., 2007). Figure 3 showed that increase

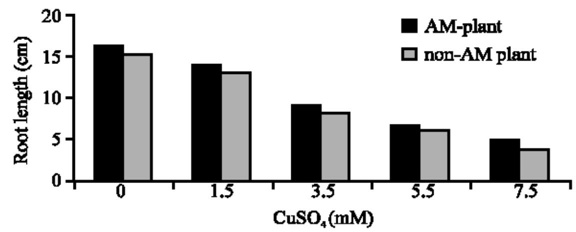

Fig. 1: Effect of $CuSO_4$ on root growth of mycorrhizal and non-mycorrhizal tomato plants

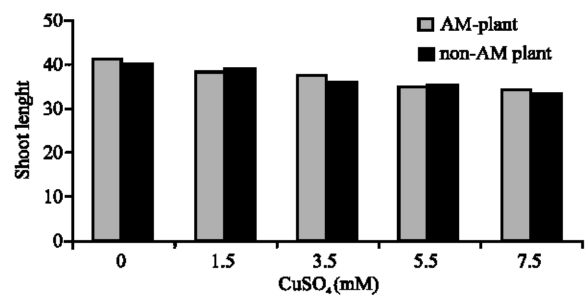

Fig. 2: Effect of $CuSO_4$ on shoot growth of mycorrhizal and non-mycorrhizal tomato plants

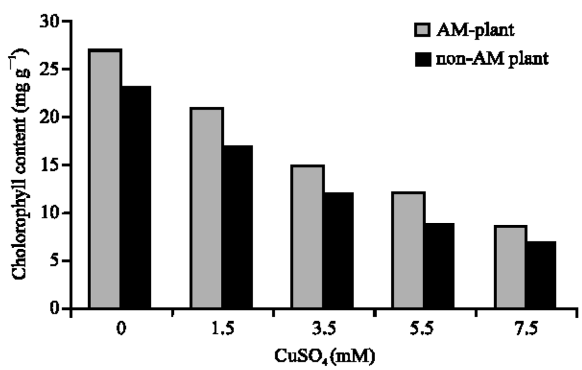

Fig. 3: Chlorophyll content of Am and non-Am plants grown in soil with Cu concentration

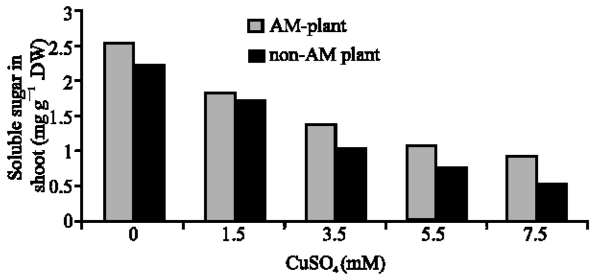

Fig. 4: Soluble sugar content in shoot of mycorrrhizal and non mycorrhiozal lettuce plants

the concentration in Cu, reduced the chlorophyll content in both AM and non- AM plants. Figure 4 showed that with increase in Cu concentration in nutrient solution, the content of total sugar was decreased.

DISCUSSION

Figure 1 showed that the effect of $CuSO_4$ on the root growth of AM and non-AM tomato plants. Increase of $CuSO_4$ concentration decreased root growth, progressively. But AM root had higher length than non-AM roots. However, no reduction of shoot length was observed (Fig. 2).

Despite the different mobility of metal ions in plants, the metal content is generally greater in roots than in the above-ground tissues (Parviz Malekadeh *et al*., 2007). In most environmental conditions, Cd enters first the roots and consequently they are likely to experience Cd damage first (Sanitá di Toppi and Gabrielli, 1999).

Dang *et al*. (2004) reported that species able to accumulate relatively high metal concentrations in aboveground tissues could be good candidates for phytoextraction. These results suggested that the AM fungi acts in tomato plants as an heavy metal filter to maintain low heavy metal concentrations in aboveground plant tissues. In this connection, Dang *et al*. (2004) reported that plant species which have strong ability to reduce metal translocation from roots to shoots are suitable as phytostabilizers for revegetation of metal contaminated lands.

Figure 3 showed that increased in $CuSO_4$ concentration decreased chlorophyll content.

Van Thichelen *et al*. (2001) showed that some mycorrhizal species protect *Pinus sylvestris* against Cu toxicity extracellularly, although the amount of Cu retained by different fungi vary considerably. The mechanisms employed by the fungi are probably through binding to extracellular materials.

Figure 4 showed that increased in $CuSO_4$ concentration decreased total sugar content. Symbiotic interactions in AM associations are based on the exchange of carbohydrates and mineral nutrients between the plant and the fungus. Wright *et al*. (1998a) using mycorrhizal and non-mycorrhizal clover plants of comparable plant size and growth rate and with similar N and P contents, demonstrated that AM fungal colonization stimulated the rate of photosynthesis sufficiently to compensate for the carbon requirement of the fungus and to eliminate growth reduction of the autotroph. The consumption of carbon by AM fungi can be up to 20% of the host photosynthate (Harris *et al*., 1985; Jakobsen and Rosendahl, 1990). Therefore plant roots become strong sink for carbohydrates when colonized by AM fungi (Wright *et al*. 1998b). In conclusion, the requirement for carbohydrates by AM fungi could cause an increased allocation to an accumulation of soluble sugars in the roots. This higher accumulation of soluble sugars in mycorrhizal plant tissue, special in roots could make mycorrhizal plants more resistant to copper stress induced by exposure to copper toxicity.

In the present study, mycorrhizal acquisition may account for a high proportion of Zn, Cu tomato plant tissues that grew at heavy metal contaminated soil. The higher heavy metal concentration in AM plants could be explained by the fact that AM infection increased plant uptake of metals by mechanisms such as enlargement of the absorbing area, volume of accessible soil and efficient hyphal translocation (Yu *et al*., 2004). In addition, although heavy metal concentrations in AM plants were much higher than that in non-AM plants, some metal toxicity (data not shown) was observed only on non-AM plant. This result suggests that AM infection offers some protection against metal toxicity. Most reports note a positive effect of am inoculation on the growth of plants in metal-contaminated soils. This protective benefit may be related to the adsorptive or binding capability for metals of the relatively large fungal biomass associated with the host plant roots, which may physically minimize or exclude the entry of metals into host plant (Cairney and Meharg, 1999). Protective responses of Am fungi to metal toxicity among AM plants have been variable, but generally existent, depending on host plant and fungal isolate sources (Andrad *et al*., 2004).

Exclusion of heavy metals was suggested as a tolerance strategy by AM tomato plants.

These results suggested that the Am fungi acts in tomato plants as an heavy metal filter to maintain low heavy metal concentrations in aboveground plant tissues.

ACKNOWLEDGMENTS

The authors are grateful to Sanam, Fradieh and Zeinab Malekzadeh for commenting on the English.

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