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Study of effect of arbuscular mycorrhiza (*Glomus intraradices*) fungus on wheat under nickel stress

A. Heydarian[1], H.R. Tohidi Moghadam[1], T.W. Donath[2] and M. Sohrabi[2,*]

[1]Islamic Azad University Varamin- Pishva Branch, Department of Agronomy, IR 33817-74895 Varamin, Iran
[2]Kiel University, Department of Landscape Ecology, Institute for Natural Resource Conservation, Olshausenstr. 75, DE24118 Kiel, Germany
[*]Correspondence: msohrabi@ecology.uni-kiel.de

Abstract. In many regions of the world soils are contaminated with heavy metals and therefore restricted in their use. For instance, the absorption of nickel (Ni) in the tissue of plants increase the plant's metabolism and cause physiological disorders or even death. Arbuscular mycorrhizal fungi are known to enhance the tolerance of host plants to abiotic and biotic stress. Thus, we investigated the potential of the arbuscular mycorrhizal fungi *Glomus intraradices* to mitigate deleterious effects of Ni in wheat. The experiment was conducted using four levels of Ni (0, 60, 120 and 180 mg per kg of soil) and two levels of mycorrhizal fungi application (with and without *Glomus intraradices*). Nickel stress significantly decreased seed number per spike, thousand-seed weight, seed yield per plant, concentration of chlorophyll a and b. At the same time, we found increased catalase (CAT) enzyme activity and dityrosine (DT) treatments. Mycorrhizal fungi application attenuated Ni effects, i.e. fungal presence increased seed number per spike, thousand-seed weight, chlorophyll a and b. Furthermore mycorrhizal fungi application reduce CAT enzyme activity and DT. In general, our results suggest that mycorrhizal fungi application reduces harmful effects of Ni stress in wheat.

Key words: CAT, chlorophyll, dityrosine, mycorrhizal fungi, nickel.

INTRODUCTION

Soil contamination with heavy metals as a result of human activities such as mining, metallurgical processes and application of fertilizers, pesticides and fungicides in agriculture is a serious threat for ecosystems and human health. Particularly when food crops are grown on contaminated soil, heavy metals may enter the human food chain (Dixi et al., 2001; Sheetal et al., 2016). The transfer and accumulation of heavy metals in soil-plant systems is impress by multiple factors (Wang et al., 2017). Here we test, whether mycorrhizal fungi may be used to reduce the heavy metal uptake by wheat and thus may pose a possibility to grow crops on heavy metal contaminated soils without challenging human health. Among the heavy metals, Nickel (Ni) is an essential micronutrient for plant growth and development (Eskew et al., 1983). However, it becomes toxic at high concentrations, Excess Ni disturbs photosynthesis and membrane function (Moya et al., 1993; Madhava Rao & Sresty, 2000; Boominathan & Doran,

2002). High condensation of Ni, excite the production of reactive oxygen species (ROS) such as superoxide ion (O^{2-}) and hydrogen peroxide (H_2O_2) at cellular level. (Gajewska & Sklodowska, 2007) and caused oxidative stress by membrane lipid peroxidation (Baccouch et al., 1998). The most obvious symptoms of Ni-toxicity, the inhibition of growth, chlorosis, necrosis and wilting have been reported not only for wild but also crop plants such as cabbage or wheat (Pandey & Sharma 2002; Gajewska et al., 2006). The symbiosis between plants and arbuscular mycorrhiza (*Glomeromycota*) is one of the most substantial interactions between plants and microorganisms in the soil For the plant this coexistence leads to strengthened absorption of nutrients such as phosphorus, nitrogen and micronutrients through mycelium (Kapulnik & Douds, 2000; Javaid, 2009) and in exchange host plants provide carbohydrates to the fungi (Smith & Read, 2008). An enhanced phosphorus concentration in plants, in turn, raises photosynthesis rate and carbohydrates production (Parádi et al., 2003). Yet, arbuscular mycorrhizal fungi not only assist host plants in absorbing nutrients, but also improve their tolerance against environmental abiotic factors such heavy metals (Jahromi et al., 2008).

An improved tolerance against heavy metals is achieved by arbuscular mycorrhizal fungi binding heavy metals to their cell walls (Hildebrandt et al., 2007) and emit glomalin (Gonzalez-Chavez et al., 2004). Moreover, mycorrhiza fungi participate in nontoxic formation through symbiosis with plants to accumulate heavy metals in plant root (Joner & Leyval, 1997). The performance of these fungi in soils contaminated with heavy metals have been fulfilled by several studies (Khan et al., 2000; Abdel Latef, 2011; Miransari, 2011; Abdel Latef, 2013). In general, mycorrhizal fungi improve mineral nutrient balance, especially rare nutrients, stimulate their uptake when the nutrients amount is low and inhibit their absorption when amount of nutrients is high. The aim of this study was to determine Ni distribution in shoot and root of wheat grown under Ni contaminated soil, and to understand if mycorrhizal fungi application presents a potential strategy for immobilizing Ni, thus reducing its deleterious effects in wheat. We particularly hypothesized that the application of the arbuscular mycorrhizal fungi *Glomus intraradices* to wheat (i) lowers the inhibition of seed germination as well as, (ii) growth and development, and (iii) the decrease in yield of wheat when grown in nickel contaminated soil.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at Varamin- Pishva, Iran, in 2016. It followed a completely randomized 4 × 2 factorial design, with three replicates per treatment combination. The soil of the experimental site was a clay loam one, with a montmorillonite clay type, low in nitrogen (0.06–0.07%), low in organic matter (0.56–0.60), and alkaline in reaction with pH of 7.2 and Ec = 0.66 dS m^{-1}. The soil texture was sandy loam, with 10% of neutralizing substances. For the experiment, sterilized field soil (autoclaved at 121 °C and pressure of 15 atmospheres for one hour , before being used as culture soils) was used to prepare four levels of Ni-contamination (0, 60, 120 and 180 mg of Ni chloride per 1 kg of soil) and filled in 30 x 30 cm plastic pots. For the second treatment factor, the top layer of the soil of half of the pots was inoculated with 2 g of Glomus intraradices spores and fungal propagules inoculum (purchased from Biotech Turan Company, Semnan, Iran) just before seed sowing. Each pot was fitted with ten seeds of cultivar Pishtaz before placing it in the controlled environment. The

room was equipped with cool white fluorescent lamps. Room air temperature was 20–22 °C, during the 16/8 h light/dark photoperiod. Photosynthetically active radiation at the top of the canopy was 400 μmol m^{-2} s^{-1}, during the light photoperiod. Relative humidity in the room was 70%. Plants were hand irrigated daily until optimal field capacity was reached. The field capacity was determined by slowly saturating four soil-filled pots until water started to drip from the bottom. Pots were covered and allowed to drain for 24 h and then weighted. The average weight was the definition of field capacity and the pots were kept watered to this weight. At seed filling stage, flag leaves were collected and immediately frozen in liquid nitrogen and stored at 80 °C until further laboratory analyses. At physiological maturity stage, plants were harvested at the soil surface and seed per spike counted and weighted to determine thousand seed weight and seed yield per plant. Chlorophyll was extracted by 80% acetone according to Arnon (1949). The extracts were filtrated, and chlorophyll a and b content were determined by spectrophotometer (model Cintra 6 GBC; GBC Scientific Equipment, Dandenong, Victoria, Australia) at 645 nm and 663 nm. The content of chlorophyll was expressed as mg × g^{-1} of fresh weight. Catalase activity was estimated by the Cakmak & Horst (1991) method. The reaction mixture contained 100 μl of crude extract, 500 μl of 10 mM H_2O_2 and 1,400 μl of 25 mM sodium phosphate buffer. Catalase activity was estimated by recording the absorbance reduction at 240 nm, for 1 min, using a spectrophotometer.

Dityrosine was estimated by the Amado et al. (1984) method. Leaf samples were homogenized with 5 mL of 0.16 M Tris-phosphate, pH 7.5. The plant tissue homogenate was centrifuged at 5,000 g for 60 min to remove debris. o,o-dityrosine was recovered by gradient elution from the C-18 column (Econosil C18, 250 mm × 10 mm) and was analyzed by reversed-phase HPLC with simultaneous UV-detection (280 nm). A gradient was formed from 10 mM ammonium acetate, adjusted to pH 4.5 with acetic acid, and methanol, starting with 1% methanol and increasing to 10% over 30 min. A standard dityrosine sample was prepared according to Amado et al. (1984). Dityrosine was quantified by assuming that its generation from the reaction of tyrosine with horseradish peroxidase in the presence of H_2O_2 was quantitative (using the extinction coefficient e315 = 4.5 mM^{-1} cm^{-1} at pH 7.5). From each treatment, one gram of dried tissue (root and shoot) was placed in an electrical oven at 480 °C for 5 h. After cooling, obtained ash was solved in 10 mL 10% nitric acid. After filtering, the solution was poured in plastic tubes and the amount Ni in roots and shoots were analyzed by atomic absorption spectroscopy (Model GBC 932 plus) (Reeves et al., 1996).

All data were analyzed by analysis of variance using the GLM procedure in SAS (SAS Institute Inc., 2002). The assumptions of the variance analyses were tested by checking if the residuals were random, homogenous, with a normal distribution and a mean of about zero. The significance of differences among means was carried out using Duncan's multiple range test at $p < 0.05$.

RESULTS AND DISCUSSION

The main effects of Ni and fungus were significant on all measured traits (Table 1). The interaction between Ni and fungus was significant on CAT enzyme activity (Table 1). The lowest seed number per spike, 1,000 seed weight and seed yield per plant were obtained when wheat plants were exposure to 180 mg $NiCl_2$ (Table 2).

Table 1. Analysis of variance on wheat attributes affected by nickel stress and mycorrhizal fungi

S.O.V	d.f	Seed number per spike	Thousand seed weight	Seed yield per plant	Chlorophyll a	Chlorophyll b	C a
Ni	3	55.38**	65.40**	44.57**	0.205**	0.003**	1
Myc	1	5.22**	91.26**	6.38*	0.035**	0.005**	2
Ni* Myc	3	0.21ns	2.27ns	0.35ns	0.0001ns	0.00001ns	2
Error	16	0.47	6.76	1.69	0.0003	0.00006	5
C.V (%)		1.73	6.77	6.02	1.46	1.61	5

*, ** and ns significant at 0.05, 0.01 percentage and no significant, Myc = Mycorrhiza, Ni = nickel.

Table 2. Comparison of main means wheat attributes affected by nickel stress condition and mycorrhizal fungi

Treatment	Seed number per spike	Thousand seed weight (g)	Seed yield per plant (g)	Chlorophyll a (mg g^{-1}FW)	Chlorophyll b (mg g^{-1}FW)	Cat act (ΔA
Nickel concentration						
0	43.31a	42.15a	11.04a	1.576a	0.540a	74.
60	41.58b	40.11a	9.51a	1.368b	0.500b	116
120	37.65c	36.36b	7.14b	1.236c	0.495b	170
180	37.05c	35.00b	4.82c	1.151d	0.485c	187
Mycorrhiza						
-	39.43b	36.45b	7.61b	1.295b	0.490b	147
+	40.36a	40.35a	8.64a	1.371a	0.520a	127

Treatment means followed by the same letter within each common are not significantly different (P < 0.05) according to Dunca

Plants grown in Ni contaminated soil showed visible symptoms of injury reflected in terms of chlorosis, growth inhibition and browning of root tips. The lowest seed number per spike, 1,000 seed weight and seed yield per plant were obtained when no mycorrhizal fungus was applied, while the highest seed number per spike, 1,000 seed weight and seed yield per plant were obtained by treating the soil with mycorrhizal fungus (Table 2). Increased water and nutrients absorption after the application of mycorrhizal fungus may explain the increase in seed number per spike, thousand seed weight and seed yield per plant. In addition, arbuscular mycorrhizal fungi may also increase plant growth by improving nutrition through increasing water uptake and reduced soil compaction (Gaur & Adholeya, 2004). The present study showed that chlorophyll a and b production decreased when wheat plants were exposure to 180 mg $NiCl_2$ (Table 2). The plants response to Ni in the soil seems to be closely linked to the chlorophyll activity, since the highest levels of chlorophyll-a and chlorophyll-b were obtained when the plants were colonized with mycorrhizal fungus (Table 2 and Fig. 2). Analogously, several authors report decreased chlorophyll contents in the leaves of Ni-exposed plants (Pandey & Sharma, 2002; Seregin & Kozhevnikova, 2006; Gajewska & Sklodowska, 2007). Dhir et al. (2009) assumes that this decline in chlorophyll levels might be due to a lower Fe content, reduced efficiency of enzymes involved in chlorophyll biosynthesis and replacement of central Mg^{2+}-molecules in chlorophyll by heavy metals. Accordingly, earlier research already suggested that some heavy metals disable the biosynthesis of chlorophyll (Ouzounidou, 1995).

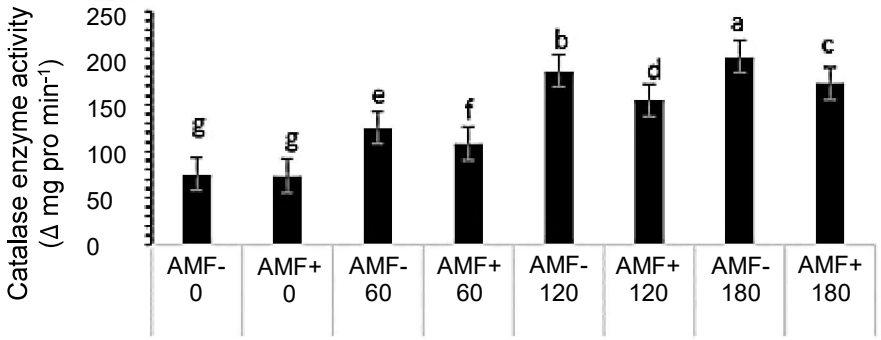

Figure 1. Interaction between nickel stress condition and mycorrhizal fungi on CAT enzyme activity- Nickel-chloride concentrations (0, 60, 120 or 180 mg per kg of soil) and presence (AMF+) or absence (AMF-) of arbuscular mycorrhizal fungi. Presented are the mean ± SE.

Sohrabi et al. (2018) also reported that the Pb-contamination caused a significant decrease in total chlorophyll contents. The lowest CAT enzyme activity was obtained when mycorrhizal fungus was applied, also according to our results the highest CAT enzyme activity was obtained when wheat plants were exposure to $NiCl_2$ concentration with 180 mg per kg of soil (Table 2 and Fig. 1). The results showed that the highest dityrosine content was observed in wheat plants exposed to 180 mg $NiCl_2$ per kg of soil (Table 2). CAT enzyme activity increases when plants have to increase their defense activity against oxidative stress. Consequently, several researchers have reported an increase in CAT activity under heavy metal stress, e.g. Cd, Hg, Ni, Pb and Fe (Ma, 2000; Pang et al., 2001; Yang et al., 2001; Parlak, 2016). The primary response of plants to

heavy metal stress is the generation of ROS upon exposure to high levels of heavy metals that which destroys chlorophyll molecules by ROS, reducing photosynthesis and growth (Wojtaszek, 1997; Halliwell & Gutteridge, 1998; Feda et al., 2004; Mithofer et al., 2004). Again, stress through heavy metals was – as indicated by a low CAT enzyme activity – lowest when mycorrhizal fungus was applied (Table 2 and Fig. 1). The capability of arbuscular mycorrhiza to lower stress plants experience when exposed to heavy metals relies on the fungi's ability to produce Glomalin (Arriagada et al., 2005). This insoluble glycoprotein, which is produced by the hyphae, was shown to bind potentially toxic elements including heavy metals (Gonzalez-Chavez et al., 2004).

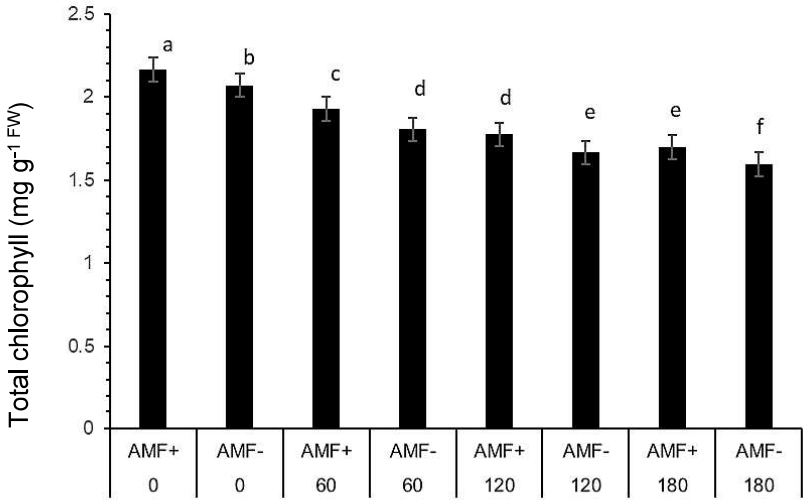

Figure. 2. Interaction between nickel stress condition and mycorrhizal fungi total chlorophyll (mg $g^{-1\,FW}$) activity- Nickel-chloride concentrations (0, 60, 120 or 180 mg per kg of soil) and presence (AMF+) or absence (AMF-) of arbuscular mycorrhizal fungi. Presented are the means ± SE.

CONCLUSION

In conclusion, evidence is now accumulating that mycorrhizal fungus can filter out toxic heavy metals and thus keep them away from the plants. The results of this study showed that inoculation with Glomus. intraradices increases wheat resistance against Ni stress and improves seed yield under Ni stress.

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