Effects of Inoculation with Rhizospheric *Pseudomonas* on Physiological Responses in the Broad Bean (*Vicia Faba*) Grown Under Copper Stress

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Abstract

The accumulations of chemical pesticides residues in soils represent one of the greatest risks for the environment and especially for plants. The copper bioviability in rhizosphere is the clearest aspect of this pollution process, causing high levels of metal stress, reducing crops yield by altering physiological and biochemical processes in plants. To face such risks, researchers have frequently studied the effect of plant growth promoting rhizobacteria (PGPR) as enhancer of the plant growth under abiotic stress. The goal of this study was firstly to know the level of broad bean tolerance to metal stress of copper, and its physiological effects in the plant, on the one hand, and the role of plant-*Pseudomonas* interaction in enhancing the plant tolerance level to metal stress, on the other. In this study, three *Pseudomonas* strains were isolated and screened by salinity and copper tolerance, then they were individually used as an inoculum in rhizosphere of broad bean *Vicia faba* (OTONO variety) in the presence of 0; 2.5; 10 and 20 $mM.L^{-1}$ of $CuSO_4$. According to the obtained results, under copper stress conditions with and without bacterial inoculation, the production of biomass and total chlorophyll content were significantly decreased. Copper treatments increased proline content in inoculated plants with P7 and P15 strains and in those which were not inoculated. However, this content was decreased in inoculated plants with P1 strain. The inoculation with P1, P7 and P15 strains motivated the production of fresh biomass and accumulation of proline, and otherwise decreased total chlorophyll content in plants.

Keywords

Copper stress • *Pseudomonas* • PGPR • *Vicia faba* • Proline • Chlorophyll

1 Introduction

Heavy metals considerably affect the physiological and biochemical processes of the plant, metal stress reduces also crop productivity. Researchers have frequently studied the effect of plant growth promoting bacteria (PGPR) as promoter under abiotic stress. In this study, the goal was firstly to know the level of broad bean tolerance to stress induced by metal stress of copper in soil, and manifested responses in the plant. The second purpose was to find out the role of plant-*Pseudomonas* interaction in improving the tolerance level of plant to metal stress of copper.

2 Materials and Methods

After preparing the bean seeds for germination and the inoculation solution from the tree screened strains, the first inoculation was applied simultaneously with the transplantation of the germinated seeds by adding 120 mL of the bacterial suspension to each pot. The second inoculation was carried out four weeks after transplantation. After one week of transplanting, the pots were regularly irrigated by the HOAGLAND solution. From the fifth week of transplantation, the irrigation solutions (HOAGLAND solution) are provided by $CuSO_4$ treatments during three weeks, at different doeses: 2.5 $mM.L^{-1}$, 10 $mM.L^{-1}$ and 20 $mM.L^{-1}$.

The plantation was carried out in a greenhouse at the Agronomy Workshop in Mazzagran, Abdelhamid Ibn Badis University in Mostaganem located at latitude: 35° 53′31.9″ N; longitude: 0° 5′7.4″E with an average temperature of 28 °

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C in the day and 23 °C at night, and a hygroscopy of 55–75%. The experiment was carried out with 16 pots with 4 seeds per pot and 4 pots for each inoculation treatment. The used bean seeds were from Otono variety. They were germinated after their disinfection with a 25% sodium hypochlorite solution for 15 min and then transplanted into pots of 5 kg of mineralized and sterile sand.

The experiment was carried in a completely randomized design with three replications. The data analysis ANOVA was carried out by STAT BOX 6.40. Means were compared and tested for significance using Student–New mean-Keuls test, at 0.05 level of probability.

3 Results and Discussion

The results show that 2.5, 10 and 20 Mm copper treatments affected proportionally the fresh weight of inoculated and non-inoculated *Vicia faba* plants. Lewis et al. (2001) reported that the excess of copper in the soil plays a toxic role and induces damage to the plant. This leads to growth retardation and leaf chlorosis. The total weight of plant biomass decreases in the presence of copper (Oliveira et al. 2014).

However, the inoculation with P1, P7 and P15 strains increased significantly the fresh weight of inoculated plants in the presence as in absence of copper treatment comparatively with the non-inoculated plants. This result is similar to those of some authors who stated that heavy metal-resistant bacteria isolated from a rhizosphere can be used to support plant growth and increase the accumulation of heavy metals (Andreazza et al. 2010; Chen et al. 2008; Dell'Amico et al. 2008; Kumar et al. 2008; Sheng et al. 2008; Ma et al. 2009). This is due to the bioavailability of copper in soil in the presence of bacterial inoculum (Oliveira et al. 2014) (Fig. 1).

3.1 Total Chlorophyll Content

The results obtained, in the absence of bacterial inoculation, show that the total chlorophyll in the leaves of plants is proportional to the copper concentration, indicating the role of copper in the synthesis of photosynthetic pigments. These results have previously been confirmed by many studies. Chugh and Sawhney (1999) suggested that low concentrations of copper increased the chlorophyll content (a) in the plant of *Elsholtzia splendens*, while this content was reduced by high concentrations. But the chlorophyll (b) content is less influenced by high or low concentrations of Cu (Li et al. 2003). Copper can increase or decrease leaf chlorophyll and alter chlorophyll fluorescence and photosystem II activity (Cook et al. 1997; Pätsikkä et al. 2002).

However, low levels of total chlorophyll content were caused by P1, P7 and P15 strains inoculation, in the presence and absence of copper treatments. This decrease is probably due to the hyper-accumulation of copper affecting chlorophyll. This hyperaccumulation of copper is caused by bacterial inoculation, increasing the bioavailability of copper in the soil. Several previous studies have suggested this effect of bacteria on the bioavailability and bioaugmentation of metals via the production of siderophores considered as chelating agents of metals (Kumar et al. 2008; Sheng et al. 2008; Egamberdiyeva 2007) (Fig. 2).

3.2 Proline Content

The results of proline contents in non-inoculated plants show a significant increase in the proline induced by the copper treatment (10 and 20 mM.L^{-1}), indicating the effect of copper stress. This observation has been reported by several studies on different plant species. High level of copper may be one of the stressors and may induce stress accumulation in plant

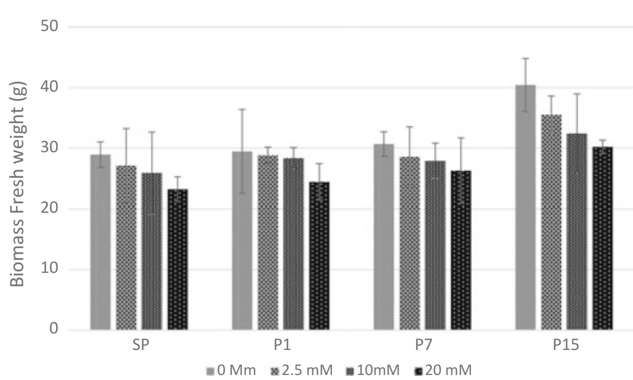

Fig. 1 Combined effect of copper and inoculum on Biomass fresh weight of plant; SP: plant without inoculation; P1, P7 and P15: Treatment with inoculum strains; 0, 2.5, 10 and 20 mM: Copper treatment

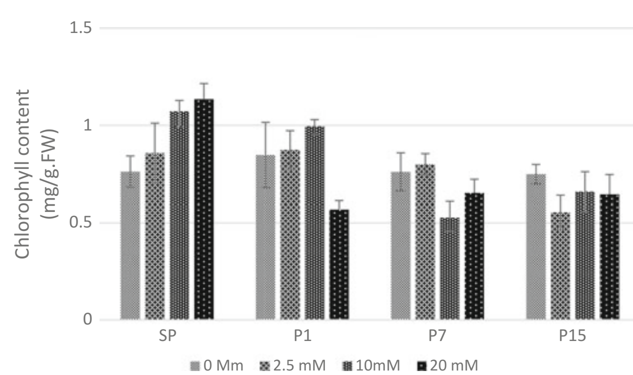

Fig. 2 Combined effect of copper and inoculum on total chlorophyll content in aerial part; SP: plant without inoculation; P1, P7 and P15: Treatment with inoculum strains; 0, 2.5, 10 and 20 mM: Copper treatment

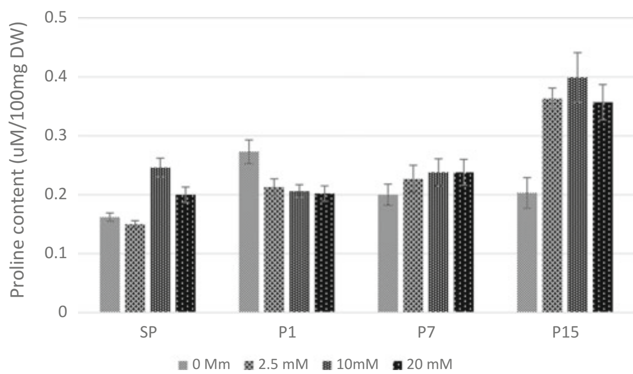

Fig. 3 Combined effect of copper and inoculum on the proline content; SP: plant without inoculation; P1, P7 and P15: Treatment with inoculum strains; 0, 2.5, 10 and 20 mM: Copper treatment

tissues (Oves et al. 2013; Zhang et al. 2008; Joseph et al. 2007; Chen et al. 2001; Miller et al. 2005; Kavi et al. 2005; Alia 2008; Alia and Matysik 2001; Ibrahim et al. 2011). Excess of CuSO4 induced proline accumulation in *C. reinhardtii* (Zhang et al. 2008), and sunflower (Zengin and Kirbag 2007).

However, in the presence of inoculation with P7 and P15, the copper treatments caused a significant increase in proline. This is probably due to the hyper-accumulated copper content in the plant tissues, as shown in many similar studies (Andreazza et al. 2010; Kumar et al. 2008; Sheng et al. 2008). This might be also due to the inoculation strains by stimulation of proline production in the plant organs (Zarea et al. 2012). This effect is significantly clear in inoculated plants with P1. and P15 and were not treated with copper (Fig. 3; Table 1).

4 Conclusions

The results showed that applied copper treatments affected proportionally the decrease of *Vicia faba* biomass, however, the P1, P7 and P15 inoculum has increased the plants biomass. An increase of total chlorophyll content in non-inoculated *Vicia faba* plants was shown; however, the levels of total chlorophyll in the presence of inoculation have significantly decreased. We also found that the effect of copper on proline was distinguished among the inoculation strains, whereas, all the inoculation treatments caused an increase in proline comparatively with non-inoculated plants.

Table 1 Combined effect of copper and inoculum on biomass fresh weight, proline ant chlorophyll content

Bacterial inoculum	Copper treatment (Mm)	Fresh weight (g)	Proline content (uM/100mg DW)		Chlorophyll content (mg/g FW)	
SP	0	28.908 ± 2.107	0.162 ± 0.007	e	0.761 ± 0.081	cdef
SP	2.5	27.103 ± 6.105	0.150 ± 0.006	e	0.857 ± 0.154	bcd
SP	10	25.898 ± 6.721	0.246 ± 0.016	cd	1.072 ± 0.056	a
SP	20	23.193 ± 2.048	0.200 ± 0.013	d	1.135 ± 0.08	a
P1	0	29.443 ± 6.929	0.273 ± 0.02	c	0.847 ± 0.168	bcdef
P1	2.5	28.805 ± 1.361	0.213 ± 0.014	d	0.873 ± 0.099	bc
P1	10	28.335 ± 1.748	0.206 ± 0.011	d	0.995 ± 0.034	ab
P1	20	24.42 ± 3.011	0.202 ± 0.013	d	0.569 ± 0.043	hg
P7	0	30.658 ± 2.036	0.200 ± 0.018	d	0.76 ± 0.098	cdef
P7	2.5	28.547 ± 4.953	0.227 ± 0.023	d	0.799 ± 0.055	cde
P7	10	27.895 ± 2.928	0.238 ± 0.023	cd	0.527 ± 0.082	g
P7	20	26.27 ± 5.412	0.238 ± 0.022	cd	0.652 ± 0.07	defg
P15	0	40.418 ± 4.398	0.203 ± 0.026	d	0.748 ± 0.05	cdef
P15	2.5	35.497 ± 3.093	0.363 ± 0.018	b	0.554 ± 0.086	g
P15	10	32.418 ± 6.524	0.399 ± 0.042	a	0.658 ± 0.103	defg
P15	20	30.202 ± 1.105	0.357 ± 0.03	b	0.644 ± 0.102	efg

Value Average ± standard deviation; Lowercase letter express groups of compared means using the Newman—Keuls test with the value of $P = 0.05$

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