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Mitigation of Cu stress by legume-*Rhizobium* symbiosis in white lupin and soybean plants

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ABSTRACT

The effect of *Bradyrhizobium*-legume symbiosis on plant growth, toxicological variables and Cu bioaccumulation was studied in white lupin and soybean plants treated with 1.6, 48, 96 and 192 μ M Cu. In both species, those plants grown in the presence of root nodule-forming symbiotic *Bradyrhizobium* showed less root and shoot growth reduction, plus greater translocation of Cu to the shoot, than those grown without symbiotic *Bradyrhizobium*. The effective added concentrations of Cu that reduced shoot and root dry weight by 50% (EC_{50}), and the critical toxic concentration that caused a 10% reduction in plant growth ($CTC_{10\%}$), were higher in plants grown with symbiotic *Bradyrhizobium*, and were in general higher in the roots whether the plants were grown with or without these bacteria. The production of malondialdehyde and total thiols was stimulated by Cu excess in the shoots and roots of white lupin grown with or without symbiotic *Bradyrhizobium*, but mainly in those without the symbionts. In contrast, in soybean, the increases in malondialdehyde and total thiols associated with rising Cu concentration were a little higher (1.2-5.0 and 1.0-1.6 times respectively) in plants grown with symbiotic *Bradyrhizobium* than without. Finally, the organ most sensitive to Cu excess was generally the shoot, both in white lupin and soybean grown with or without symbiotic *Bradyrhizobium*. Further, *Bradyrhizobium*-legume symbiosis appears to increase the tolerance to Cu excess in both legumes, but mainly in white lupin; plant growth was less reduced and $CTC_{10\%}$ and EC_{50} values increased compared to plants grown without symbiotic *Bradyrhizobium*. *Bradyrhizobium* N_2 fixation in both legumes would therefore seem to increase the phytoremediation potential of these plants when growing on Cu-contaminated sites.

26 *Keywords:* Copper excess; *Glycine max* L.; Legume-*Rhizobium* symbiosis; *Lupinus*
27 *albus* L.; Nitrogen fixation; Phytoextraction

28 *Abbreviations:* BAF, bioaccumulation factor; CTC_{10%}, critical toxic concentration of Cu
29 causing a 10% reduction in plant growth; Cu, copper; DW, dry weight; EC₅₀, the
30 effective concentration of Cu that reduces shoot or root dry weight by 50%; FW, fresh
31 weight; MDA, malondialdehyde; N, nitrogen; ROS, reactive oxygen species; SB,
32 symbiotic *Bradyrhizobium*; -SH, total thiols

33

1. Introduction

Nitrogen (N) availability often limits plant growth. Most plants obtain their N from the soil, largely via fertilizers or mineralised indigenous organic matter. However, other plants, most notably legumes, can obtain N from atmospheric N₂ via the entry of their roots into an endosymbiotic association with N₂ fixing bacteria (Stougaard, 2000). This association is the main N-input process in natural ecosystems. It is also of great importance to agriculture since it provides a more effective, cheaper and cleaner way of improving soil fertility than the administration of either inorganic or organic fertilizers.

High soil concentrations of heavy metals have a detrimental effect on microbial activity, soil fertility and bacterial nitrogen fixation (McGrath et al., 1995; Giller et al., 2009), and can cause significant yield losses. Copper is an essential element for plant growth and development, playing an important role in the production of chlorophyll and certain enzymes, in protein and carbohydrate metabolism, and in symbiotic N₂ fixation. However, when it is present at leaf concentrations of 20 µg g⁻¹ dry weight (DW) it is highly toxic (Marschner, 1995). In general, Cu excess interferes with photosynthesis, pigment synthesis, plasma membrane permeability and other metabolic process, causing the strong inhibition of plant growth (Yruela, 2009). High Cu levels (>80 mg kg⁻¹) have been recorded in some natural soils, but Cu excess most frequently occurs in those polluted by Cu-rich pig and poultry slurries, fertilisers and fungicides, industrial and urban activities, metal mining and processing, and waste disposal (Kabata-Pendias and Pendias, 2001).

Contaminated soils are generally poor in nutrients and organic matter. The inclusion of N₂ fixing plants in stabilizing vegetation can, however, help in ecosystem development by increasing available soil N and by promoting plant cover (Frérot et al.,

2006). The use of legumes for improving the fertility of soils contaminated with trace elements is therefore of great interest (Frérot et al., 2006). Soybean is the most economically important of all grain legumes. It is the main protein source in countries (e.g., China) with significant soil trace element (such as As and Cu) contamination, and is used as a model system for legume–*Rhizobium* investigations (Reichman, 2007). White lupin is a temperate grain legume of great agronomic potential due to its high seed protein content and positive effect on soil fertility. The ability of white lupin to survive in soils with low pH and nutrient availability, and the species' intrinsic biomass production and relative tolerance to trace elements (measured via stress indicators) such as Cd, Hg and As (Zornoza et al., 2002; Esteban et al., 2008; Vázquez et al., 2009), suggest it to be a suitable legume for use in the remediation of contaminated soils.

Plant assays are required when assessing the ecotoxicity of different contaminants in soils. Efroymson et al. (1997) developed toxicological benchmarks in terrestrial plants for contaminants of potential concern. Endpoints such as shoot length, biomass production, percentage germination and root growth are frequently used in these assays (OECD, 2004; USEPA, 1996), and some studies have shown that excess heavy metals can influence these endpoints (An, 2006; Cao et al., 2007; Lee et al., 2008). The present study attempts to determine whether *Rhizobium*-legume symbiosis renders white lupin and soybean plants more or less tolerant to Cu excess. Differences in growth, Cu bioaccumulation and toxicological variables (metabolic and non-metabolic) were assessed in both legumes grown with or without root nodule-forming symbiotic *Bradyrhizobium* (SB) and/or Cu excess under controlled conditions, in order to test the hypothesis that the symbiotic association *Rhizobium*-legume reduces the toxic effect of Cu excess. The experiments were performed under hydroponic conditions, to control the bioavailability of the metal supplied. This system allows: i) the effects of the

Cu doses supplied to plants to be observed, ii) the toxic Cu concentration for each species studied to be determined, and therefore, iii) the Cu tolerance of the different species to be compared.

2. Materials and methods

2.1. Growth conditions and Cu treatments

Seeds of white lupin (*Lupinus albus* L.) cv. Marta and soybean (*Glycine max* L.) cv. Williams were surface-sterilised in 10% v/v sodium hypochlorite for 15 min, rinsed thoroughly with deionised water and germinated on water-moistened filter paper in the dark at 28°C for 3 days. These seedlings were then transferred to plastic Riviera pots (three seedlings to each pot) containing 2 L of perlite in the upper compartment. Each of the white lupin plants was inoculated twice (at sowing and 1 week later) with a 1 mL suspension of *Bradyrhizobium* sp. ISLU-16; each of the soybean plants received similar inoculations of *Bradyrhizobium japonicum* USDA-110. All bacteria were in the exponential growth phase (10^8 - 10^9 cells per mL).

The lower compartment of the Riviera pots containing plants grown with no SB was supplied with 0.75 L of nutrient solution; the composition of this solution was that reported by [Zornoza et al. \(2010\)](#). The lower compartment of the Riviera pots containing plants with SB was supplied with 0.75 L of N-free nutrient solution; the composition of this solution was that described by [Sánchez-Pardo et al. \(2012\)](#). All plants were grown in a controlled environment chamber under the following night/day conditions: temperature 20/25°C, photoperiod 11/13 h, and relative humidity 60/40%. The photon flux density during the light period was $520 \mu\text{mol m}^{-2} \text{s}^{-1}$. Ten days after sowing, the plants were supplied with one of four Cu doses: 1.6, 48, 96 and 192 μM

CuSO₄.5H₂O. These high Cu doses were supplied to the plants since the perlite substrate retains approximately 50% of any heavy metals added (Vázquez and Carpena-Ruiz, 2005). Experiments were performed with four independent replicates (with 3 plants in each pot), following a randomised block design.

Plants were harvested 35 days after the initiation of the Cu treatments, separated into leaves, stems, roots and nodules, and each fraction weighed. They were then washed thoroughly with TWEEN 80 (0.1% v/v), and then three times with deionised water. One gram fresh weight (FW) of each fraction was frozen in liquid N₂ and stored at -76°C for analytical determinations. The remaining plant material was dried at 80°C for 3 days until a constant DW was achieved. It was then homogenised and the element contents determined.

2.2. Determination of Cu

The concentration of Cu in shoots (leaves plus stems) and roots was determined by digesting 200 mg DW of homogenised samples with a mixture of HNO₃:H₂O₂:H₂O (3:2:10, v:v:v) for 30 min at 125°C under a pressure of 1.5 kPa (Lozano-Rodríguez et al., 1995). The Cu concentration was determined by atomic absorption spectrophotometry (Perkin-Elmer Analyst 800).

The bioaccumulation factor (BAF) for Cu in the white lupin and soybean plants grown under all conditions was calculated as the ratio between the Cu concentration in each plant organ per plant, and the total Cu concentration added per plant to the nutrient solution.

$$BAF = \frac{\text{Cu concentration in plant organ } (\mu\text{g plant}^{-1})}{\text{Cu concentration in nutrient solution } (\mu\text{g plant}^{-1})}$$

2.3. Determination of malondialdehyde and total thiols

Frozen samples were homogenised to a fine powder in liquid N₂ using an ice-cooled mortar and pestle. Lipid peroxides were then determined as malondialdehyde (MDA, a cytotoxic product of lipid peroxidation normally considered as the major 2-thiobarbituric acid reacting compound) (Lozano-Rodríguez et al., 1997). Plant material (100 mg FW) was placed in 2.0 mL of TCA-TBA-HCl reagent (15% w/v TCA, 0.37% w/v TBA and 0.25 mM HCl), and the extract heated in a sand bath (90°C, 30 min). After cooling, the flocculent precipitate was removed by centrifugation at 11,000 x g for 10 min. Absorbance of the supernatant was measured at 535 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. Total thiols (-SH) were assayed using 100 mg FW of plant material with 0.4 mL of NaOH (1 M) containing NaBH₄ (1 mg.mL⁻¹) and 0.2 mL of deionised water. After centrifugation (11,000 x g, 10 min), 0.5 mL of supernatant was added to 0.5 mL of 5,5'-dithiobis(2-nitrobenzoic acid) dissolved in neutralizing buffer (0.5 M potassium phosphate, pH 7.2), and absorbance measured at 410 nm (Jocelyn, 1987).

2.4. Toxicological variables and statistical analyses

Relative shoot and root growth rates were expressed as a percentage of the growth of plants (based on FW) compared to the corresponding control treatment (An, 2004a). The critical toxic concentration of Cu causing a 10% reduction in plant growth (CTC_{10%}), and the effective added concentrations of Cu reducing shoot or root dry weight by 50% (EC₅₀), were calculated by regression analysis using SigmaPlot 9.0 software (SPSS Inc., Chicago, IL). EC₅₀ values were calculated using a four-variable logistic curve, and CTC_{10%} values using a one-variable logarithmic curve.

The data presented are the means \pm standard errors (S.E.) of four independent replicates. To ensure that the assumptions for statistical analysis were fulfilled, the equality of variances and the normality of the data were tested. Differences between means for each variable were tested for significance by one-way analysis of variance (ANOVA). Significant differences ($P < 0.05$) between treatments were sought using the least significant difference test or Duncan's test as appropriate.

3. Results and discussion

3.1. Growth

Figure 1 shows the relative growth rates of white lupin and soybean shoots and roots when grown with or without SB and exposed to different Cu doses. In both legumes, shoot growth with or without SB was generally adversely affected by Cu excess. With the 48 μM Cu dose, white lupin shoot growth was reduced by 35% and 45% when grown with and without SB respectively. In soybean these figures were 39% and 64%. The reduction was greater with increasing nutrient solution Cu concentration, especially in the plants grown without SB. As an exception to this trend, the white lupin plants treated with 192 μM Cu showed a milder reduction in shoot growth when grown without SB (59%) than with SB (71%). An increased translocation of Cu to the shoot might have occurred in plants grown with SB conditions, which might have affected the growth and development of this organ.

The relative growth rates of white lupin and soybean roots in plants grown without SB also fell with increasing Cu concentrations. However, the negative effect of Cu excess on root growth was less in plants grown with SB. Indeed, the plants grown

with 48 μM Cu showed greater relative root growth (white lupin 12%, soybean 37%) than the corresponding control plants (1.6 μM). Reductions in shoot and root growth have been reported in several crops grown under Cu excess without symbiotic bacteria, such as cucumber, sorghum, sweet corn and wheat (An, 2004b; 2006). However, in the presence of such bacteria, an increased biomass has been reported for soybean plants treated with As (Reichman et al., 2007), for *Brassica juncea* cultivated with soil from Pb-Zn mine tailings (Wu et al., 2006), and for the Cd/Zn-hyperaccumulating plant *Sedum alfredii* when cultivated with these metals under hydroponic conditions (Li et al., 2007). According to Reichman et al. (2007) the increased biomass recorded for soybean plants grown with *Bradyrhizobium japonicum* might be due to the latter stimulating the production of growth-promoting hormones. Ghorbanli et al. (1999) reported that gibberellins were able to reduce the harmful effect of Cd excess on the growth of soybean plants when grown without symbiotic bacteria. Other authors have reported *Rhizobia* isolated from stressed environments enhance the growth of plants by promoting substances such as auxins, cytokinins, abscisic acids, riboflavin and vitamins (Ahmad et al., 2012).

Table 1 shows the $\text{CTC}_{10\%}$ and EC_{50} values for the shoots and roots of plants grown under the different Cu and SB conditions. The shoot $\text{CTC}_{10\%}$ of white lupin plants grown with SB was three times that of plants grown without SB. In soybean, this increase was x2.2. Therefore, compared to plants grown without SB, both white lupin and soybean plants grown with SB need to accumulate higher Cu concentrations in their shoots for a 10% reduction in the growth of this organ to be appreciable. Fageria (2001) have reported $\text{CTC}_{10\%}$ values for the shoot of several crops: 26 mg kg^{-1} DW for upland rice, 17 mg kg^{-1} for wheat, 11 mg kg^{-1} DW for corn, and 10 mg kg^{-1} DW for common

bean and soybean. The value recorded for the last legume is similar to that obtained in the present work for soybean shoots grown without SB ($12 \text{ mg kg}^{-1} \text{ DW}$).

No significant differences were seen between the root $\text{CTC}_{10\%}$ values of either plants species grown with or without SB (in all cases these values were always higher than those recorded for the shoot). Similar results have been reported by other authors, with shoot and root $\text{CTC}_{10\%}$ values in *Phragmites australis* grown without SB recorded at 55 and $3100 \text{ mg kg}^{-1} \text{ DW}$ (Ali et al., 2002), in *Vigna unguiculata* at 20 and $66 \text{ mg kg}^{-1} \text{ DW}$ (Kopittke and Menzies, 2006), and in *Zea mays* at 21 and $170 \text{ mg kg}^{-1} \text{ DW}$ (Ali et al., 2002).

Similarly, compared to plants grown without SB, both white lupin and soybean plants grown with SB would need higher Cu concentrations in their shoots and roots before a 50% reduction in growth would be appreciable (2.1 times more for white lupin shoot, 7.5 times more for the root; 1.9 times for the soybean shoot, and 3.8 times more for the root). Further, in white lupins grown without SB, the shoot and root EC_{50} values were similar, while in soybean the root EC_{50} value was higher in the root (1.8 times) than in the shoot. In white lupin and soybean grown with SB, the EC_{50} values for the root were respectively 3 and 3.5 times higher than those recorded for the shoot (Table 1). In barley, maize, cucumber, wheat and sorghum grown without symbiotic bacteria, other authors report shoot EC_{50} values to be higher than those of the root (Ali et al., 2004; An, 2006). However, similar values were observed in *Fallopia convolvulus* by Pedersen et al. (2000). The present $\text{CTC}_{10\%}$ and EC_{50} results indicate that higher Cu concentrations are necessary if white lupin and soybean plants grown with SB are to suffer growth reductions greater than those suffered by plants grown without SB. In addition, they show that the shoots of both species are more sensitive - in general - to Cu than the roots, whether SB are present or not. Other authors have reported shoot and

root yield reductions of 10% at higher Cu²⁺ activities in *Vigna unguiculata* plants grown without SB (1.7 µM; [Kopittke and Menzies, 2006](#)) than in those grown with SB (1.0 µM; [Kopittke et al., 2007](#)).

3.2. Bioaccumulation

[Figure 2](#) shows the changes in the ratio of BAF in the shoot to the BAF in the root (BAF_{Shoot}/BAF_{Root}) in both species. The ratio in both species, grown either with or without SB, decreased with increasing nutrient solution Cu. However, the reductions in the BAF ratio were less severe from the 48 µM Cu onwards. Similar results have been reported for cucumber, maize, sorghum and wheat treated with high concentrations of heavy metals ([An, 2004a, b](#)). The shoot and root BAF ratios for both crops grown with SB and Cu excess were less than 1 (except for white lupins grown with 48 µM Cu). These results indicate that Cu is mainly accumulated in the roots. Higher Cu concentrations in the roots than shoots have been reported by other authors ([Angelova et al., 2003](#); [Jung et al., 2003](#)). However, the presence of SB increased the translocation of Cu to the shoot; the BAF ratio of both species grown with SB was higher than when grown without SB (around 2-23 times for white lupin, and 3-8 times for soybean). In soils with high tailing contents, [Wu et al. \(2006\)](#) observed an increase in the Cu concentration of *Brassica juncea* shoots when grown in the presence of symbiotic bacteria compared to those grown in their absence. In contrast, [Li and Wong \(2012\)](#) detected a reduction in Cu translocation and the bioaccumulation factor in *Sedum alfredii* when grown in the presence of symbiotic bacteria, but an increase in Cd, Pb and Zn translocation and the corresponding BAFs. According to these authors, the presence of symbiotic bacteria has different effects depending on the metal added.

3.3. Stress indicators

Copper excess-induced oxidative stress results from the direct transfer of electrons in single electron reactions, and an increase in ROS formation, leading to the disturbance of metabolic pathways (Navari-Izzo and Quartacci, 2001). The degree of injury suffered by cells depends on the rate of ROS formation and the efficiency and capacity of their detoxification and repair mechanisms. The MDA concentration provides an index of lipid peroxidation and, therefore, of oxidative stress. Thiol groups play an important role in cytoplasmic detoxification, but they are also required to counteract the harmful effects of oxidative stress (Noctor and Foller, 1998). Tables 2 and 3 show the MDA and total -SH concentrations of the leaves and roots of white lupin and soybean plants grown under the present range of experimental conditions. In general, Cu excess stimulated the production of MDA and -SH in the shoots and roots of white lupin plants grown with and without SB (indicating oxidative damage to have occurred), but especially in those grown without SB. Lipid peroxidation and an increase in -SH production was also seen in soybean leaves and roots in plants grown with SB under Cu excess. However, in the same organs of soybean plants grown without SB, the increases in MDA and -SH production promoted by Cu excess were smaller than in those grown with SB. Indeed, a reduction in the MDA content in the roots was recorded. This might indicate that N₂ fixation reduces oxidative stress, but that this is dependent on plant species since it reduced oxidative stress induction in white lupin but not in soybean plants. Some authors have observed the content of MDA to vary depending on plant species. In general, MDA values are higher in Cu-sensitive than Cu-tolerant species (Gonnelli et al., 2001; Hartley-Whitaker et al., 2001). An increase in MDA

levels in the leaves and roots is reported in *Phaseolus vulgaris* L. (Bouazizi et al., 2010) and *Alhagi camelorum* Fisch (Boojar and Tavakoli, 2010) when exposed to Cu excess. An increase in -SH compounds has been reported in roots of *Brassica juncea* grown under Cu excess (Wang et al., 2004).

4. Conclusions

In general, the shoot was more sensitive to Cu excess than the root in both legumes studied, whether grown with or without SB; the EC₅₀ and CTC_{10%} values were smaller, and the increase in MDA production were greater than in the root. *Rhizobium*-legume symbiosis appears to mitigate Cu toxicity in both legumes; it leads to smaller reductions in shoot (around 10% in white lupin and 6-25% in soybean) and root growth (around 55% in white lupin and 45% in soybean), and increases the CTC_{10%} and EC₅₀ values. In addition, it increases Cu translocation to the aerial part of the plants. However, the presence of SB appears to have a more useful effect in white lupin than soybean plants: i) the increase in CTC_{10%} and EC₅₀ in the shoots and roots is greater in this species, and ii) it is associated with less oxidative stress and therefore a smaller antioxidant response. Finally, the inoculation of white lupin and soybean plants with *Bradyrhizobium* appears to increase their phytoextraction capacity and Cu tolerance. Ensuring *Rhizobium*-legume symbiosis occurs may therefore be important in the remediation of Cu-contaminated sites (at least up to an effective dose of around 96 µM Cu) with the present plant species.

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Figure captions

Fig. 1. Shoot and root growth as a percentage of the corresponding controls in white lupin and soybean plants grown with or without symbiotic *Bradyrhizobium* and exposed to different Cu doses. Data are means \pm S.E. ($n = 4$). Different letters above the bars indicate significant differences between plants grown with and without symbiotic *Bradyrhizobium* ($P < 0.05$).

450 **Fig. 2.** Ratio of shoot BAF to root BAF in white lupin and soybean plants grown with or
451 without symbiotic *Bradyrhizobium* and exposed to different Cu doses. Each data point
452 represents the mean \pm S.E. ($n = 4$). Where no bar is visible, the S.E. is smaller than the
453 data point.
454

Table 1
CTC_{10%} and EC₅₀ values for white lupin and soybean shoots and roots in plants grown with and without symbiotic *Bradyrhizobium* and exposed to different Cu doses. Data are means ± S.E. (*n* = 4). Values in the same row followed by different letters are significantly different (*P*<0.05).

Plant parts	Toxicological variables	With symbiotic <i>Bradyrhizobium</i>	Without symbiotic <i>Bradyrhizobium</i>
		<i>White lupin</i>	
Shoots	CTC _{10%} (mg Cu kg ⁻¹ DW)	52.39 ± 1.88 ^a	17.46 ± 0.86 ^b
	EC ₅₀ (μM)	78.56 ± 1.15 ^a	38.20 ± 0.98 ^b
Roots	CTC _{10%} (mg Cu kg ⁻¹ DW)	71.43 ± 3.80 ^a	70.24 ± 4.25 ^a
	EC ₅₀ (μM)	239.90 ± 2.65 ^a	32.1 ± 3.13 ^b
<i>Soybean</i>			
Shoots	CTC _{10%} (mg Cu kg ⁻¹ DW)	27.17 ± 4.70 ^a	12.25 ± 0.35 ^b
	EC ₅₀ (μM)	82.66 ± 1.42 ^a	42.7 ± 5.23 ^b
Roots	CTC _{10%} (mg Cu kg ⁻¹ DW)	138.41 ± 17.98 ^a	103.68 ± 3.14 ^a
	EC ₅₀ (μM)	292.20 ± 9.84 ^a	76.60 ± 6.72 ^b

Table 2
Concentration of MDA and total -SH (nmol g⁻¹ FW) in the leaves and roots of white lupin plants grown with or without symbiotic *Bradyrhizobium* and exposed to different Cu doses. Data are means ± S.E. (*n* = 4). Values in the same row followed by different letters differ significantly (*P* < 0.05).

Organ		Doses of Cu (μM)			
		1.6	48	96	192
With symbiotic <i>Bradyrhizobium</i>					
Leaves	MDA	13.7 ± 1.5 ^a	20.5 ± 0.3 ^b	26.1 ± 0.6 ^c	29.8 ± 1.3 ^d
	-SH	473.9 ± 18.5 ^a	543.9 ± 1.5 ^b	537.0 ± 23.7 ^b	511.2 ± 24.5 ^{ab}
Roots	MDA	21.4 ± 0.5 ^a	23.9 ± 1.1 ^a	38.4 ± 0.7 ^b	48.2 ± 4.0 ^c
	-SH	94.7 ± 6.3 ^{ab}	63.3 ± 10.3 ^a	95.2 ± 6.5 ^{ab}	157.4 ± 8.8 ^c
Without symbiotic <i>Bradyrhizobium</i>					
Leaves	MDA	46.8 ± 2.1 ^a	85.7 ± 1.2 ^b	113.9 ± 1.9 ^c	145.9 ± 0.8 ^d
	-SH	570.2 ± 16.9 ^a	999.8 ± 38.2 ^b	1653.0 ± 16.1 ^d	1502.9 ± 24.0 ^c
Roots	MDA	49.6 ± 2.7 ^a	67.9 ± 0.8 ^b	81.2 ± 2.3 ^c	77.4 ± 3.0 ^c
	-SH	66.2 ± 3.1 ^a	195.6 ± 2.4 ^b	236.3 ± 9.2 ^c	251.2 ± 4.6 ^c

Table 3
Concentration of MDA and total -SH (nmol g⁻¹ FW) in the leaves and roots of soybean plants grown with or without symbiotic *Bradyrhizobium* and exposed to different Cu doses. Data are means ± S.E. (n = 4). Values in the same row followed by different letters differ significantly (P<0.05).

Organ		Doses of Cu (μM)			
		1.6	48	96	192
With symbiotic <i>Bradyrhizobium</i>					
Leaves	MDA	31.5 ± 3.3 ^a	84.7 ± 3.7 ^b	91.8 ± 2.1 ^b	91.3 ± 3.5 ^b
	-SH	620.2 ± 19.6 ^a	700.0 ± 46.0 ^{ab}	821.0 ± 57.1 ^{bc}	963.8 ± 80.7 ^c
Roots	MDA	24.7 ± 0.7 ^a	56.4 ± 3.6 ^b	58.4 ± 0.9 ^b	52.7 ± 1.8 ^b
	-SH	118.1 ± 4.1 ^a	114.5 ± 2.5 ^a	197.9 ± 14.7 ^b	236.6 ± 17.0 ^c
Without symbiotic <i>Bradyrhizobium</i>					
Leaves	MDA	57.8 ± 2.6 ^a	94.9 ± 0.4 ^c	92.0 ± 078 ^c	85.5 ± 2.5 ^b
	-SH	761.8 ± 6.6 ^a	806.0 ± 19.0 ^a	1017.7 ± 31.0 ^b	1376.9 ± 4.7 ^c
Roots	MDA	41.9 ± 2.1 ^c	47.7 ± 0.9 ^d	27.6 ± 1.1 ^b	15.4 ± 1.8 ^a
	-SH	193.6 ± 1.9 ^a	259.9 ± 14.9 ^b	266.3 ± 8.0 ^b	245.9 ± 6.2 ^b

Figure 1
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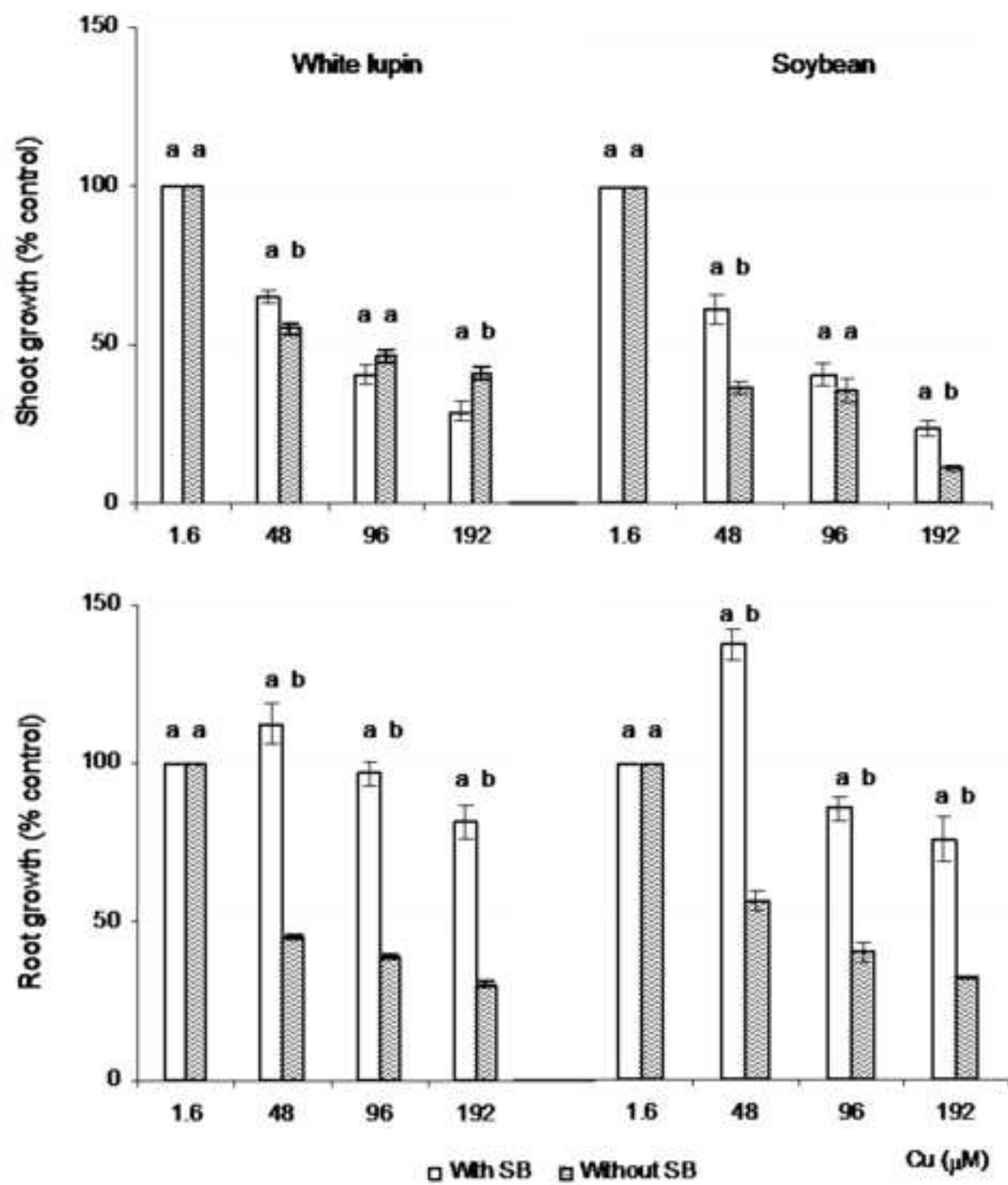


Figure 2
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