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Full Length Research Paper

Evaluating the Environmental Resilience of Salix Species: Copper and Cadmium Tolerance in North Moroccan Ecosystems

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Hydroponic culture has been used to compare copper and cadmium tolerance in five *Salix* species from North Morocco using different approaches. Measurements of growth parameters have been combined with those of some photosynthetic parameters. Tolerance index based either on root or shoot growth allowed to define *Salix pedicelata* and *Salix purpurea* as Cu and Cd-tolerant species, respectively. From the methodological point of view, the use of tolerance index based on aerial part growth, appeared most adequate for the screening of metal tolerance in woody plants. On the other hand, no significant effect on chlorophyll content was observed under treatment with either Cu or Cd, in any of the *Salix* species analyzed. *In vitro* experiments with thylakoid membranes showed that the effects on the electron transport chain differ for each metal and species used. Furthermore, it seems that metal tolerance at the chloroplast level is distinct to that of the whole plant.

Key word: *Salix*, heavy metals, tolerance index, photosynthesis.

INTRODUCTION

The use of some *Salix* species in phytoremediation for the removal of heavy metals from contaminated soils, sediments and waters (Kuzovkina and Quigley, 2005) is considered an environment friendly, cost-effective solution to remediate contaminated sites. In addition, some *Salix* clones can be harvested for the production of biomass energy (Greger and Landberg, 1999). Metal tolerance is one of the most important criteria to select an appropriate plant to remediate heavy metal contaminated sites. As *Salix* species and clones have different tolerance to particular metals (Greger and Landberg, 1999), there is a need to use a rapid screening test to determine the most tolerant species. Generally, the screening tests need sensitive indicators, providing fast and reliable information. In the present work, measurements of growth parameters have been combined to those of some photosynthetic parameters such as chlorophyll content and photosystem II (PSII) activity. Most of the studies dealing with the effect of heavy metals on photosynthesis are devoted to reactions related to PSII, describing this complex as the most sensitive to metal toxicity in the chloroplast (Arellano et al., 1994; Barón et al., 1995; Yruela et al., 2000).

MATERIALS AND METHODS

Salix cuttings (approximately 18 cm) were taken from five species (*S. pedicelata* Spe *S. babylonica* Sb, *S. eleagnos* Se, *S. purpurea*

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Abbreviations: Chl, chlorophyll; TI, tolerance index; PPBQ, phenyl-*p*-benzoquinone; PSII, photosystem II.

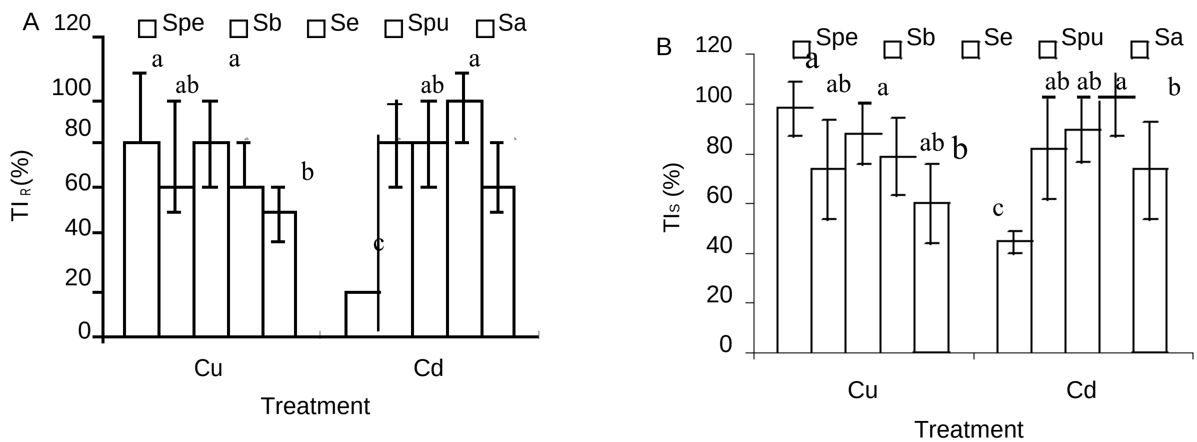

Figure 1: Tolerance index, based on root length IT_R (A) or shoot length IT_S (B), for different *Salix* species treated with 15,7 µM Cu and 44,5 µM Cd during 10 days (n=18). Spe: *S. pedicelata*, Sb: *S. babylonica*, Se: *S. eleagnos*, Spu: *S. purpurea*, Sa: *S. atrocinerea* Values followed by the same letter are not significantly different according to Duncan's test (p<0.05).

Spu, *S. atrocinerea* Sa) growing in the same area in North Morocco. Cuttings were placed for 20 days in plastic trays with water until root and shoot development. Plants were cultivated in a growth chamber at 120 µmol $m^{-2}s^{-1}$ photosynthetically active radiation (PAR), generated by a combination of Sylvania VHO cool- white fluorescent and incandescent lamps (Danvers, MA, USA), with a 16/8 h photoperiod, a temperature regime of 25ºC/20ºC (day/night) and a relative humidity of 60-70%.

Willow cuts were transferred to hydroponic culture with modified Hoagland nutrient solution (Hoagland and Arnon, 1941). When plants were placed in the hydroponic culture, copper ($CuSO_4 \cdot 5H_2O$) and cadmium ($CdCl_2$) were added at 1 mg L^{-1} (15.7 µM) and at 5 mg L^{-1} (44.5 µM), respectively. The choice of Cu and Cd concentrations used are based on preliminary multiple concentrations assays (Kohl and Losch, 1999).

Three replicates were used per treatment and 6 plants per container. All solutions were changed every 3 days to prevent depletion of metals and nutrients. The metal treatment was carried out during 10 days.

Tolerance index (TI) for the tested plants was calculated using:

TI (%) = (mean root or shoot elongation in nutrient solution with metal / mean root or shoot elongation in standard nutrient solution) x 100 (Wilkins, 1978).

Chlorophyll content from leaves and thylakoid was determined spectrophotometrically in an 80% (v/v) acetonic extract (Porra et al., 1989).

Thylakoid membranes were isolated from non-treated plants according to Arellano et al. (1994). The photosynthetic oxygen evolution of isolated thylakoid membranes was measured in the Hill reaction from water to phenyl-*p*-benzoquinone (PPBQ) (Arellano et al., 1994) with a Clark type oxygen electrode (Hansatech, Norfolk, UK). Thylakoid membranes isolated from control plants were incubated with 15.7 µM Cu and 44.5 µM Cd in the measuring solution (Arellano et al., 1994).

Statistical analysis was done by the SPSS computer program, using one way ANOVA. Comparison between species was done by Duncan's multiple range test at p<0.05.

RESULTS AND DISCUSSION

The estimation of metal tolerance at short-term exposition in herbaceous plants has been based on classical root elongation measurements (Wilkins, 1978). In this study, tolerance index was estimated considering both root and shoot elongation (Figure 1). We observed that the heavy metal impact depends on plant species and metal used for the treatment. Generally, it was more evident in roots than on the aerial part. It is well known that in plants growing in heavy metal-contaminated sites, the growth of the roots is heavily affected (Wilkins, 1978). However, a high correlation (p<0.001) has been observed in our experiments between root and shoot elongation under heavy metal treatment, with R = 0.900 and R = 0.867 for Cu and Cd, respectively. This finding is relevant from the methodological point of view, because it allowed the use of TI based on aerial growth, which it is much easier to measure.

In addition, our results have showed that *S. pedicelata* and *S. purpurea* could be described as Cu and Cd tolerant plants, respectively; while *S. atrocinerea* and *S. pedicelata* were the most sensitive to Cu and Cd, respectively. These results confirm the variability in both heavy metals tolerance and metal tolerance specificity existing between *Salix* species (Greger and Landberg, 1999).

Both Cu and Cd treatment did not influence the Chl content in any *Salix* species analysed (Table 1) . Similar results were found by Lunackova et al. (2003) in other *Salix* species treated with Cd. The toxic effects of Cu^{2+} and Cd^{2+} on oxygen evolution (Hill reaction from H_2O to PPBQ) from thylakoid membranes isolated from untreated plants are displayed in Figure 2. No significant differences were found between species for the toxicity of Cd on PSII activity. Apparently, chloroplasts from the different willow species tested were relatively tolerant at the Cd concentration used. It has been proposed that low-µM Cd^{2+} concentrations affect photosynthesis at the level of photosystem II by inhibiting oxygen evolution. However, *in vitro* studies on isolated chloroplast showed

Table 1. Total chlorophyll content (mg g^{-1}) of young leaves for different *Salix* species treated with 15,7 µM Cu and 44,5 µM Cd during 10 days (n=6). Spe: *S. pedicelata*, Sb: *S. babylonica*, Se: *S. eleagnos*, Spu: *S. purpurea*, Sa: *S. atrocinerea* ns : One way ANOVA shows not significant difference between control and metal treatment at ($p<0.05$).

Treatment	Spe	Sb	Se	Spu	Sa
Control	3.12±0.35	2.16±0.37	2.42±0.47	2.52±0.61	1.93±0.34
15.7 µM Cu	3.64±0.82	2.31±0.22	2.43±0.31	2.66±0.30	1.94±0.28
44.5µM Cd	3.33±0.37	2.09±0.21	2.21±0.44	2.51±0.25	1.78±0.21
ANOVA	ns	ns	ns	ns	ns

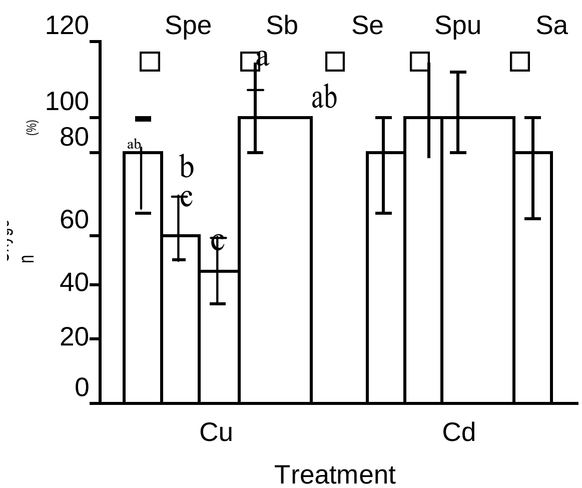

Figure 2: Effect of Cu and Cd on oxygen evolution of thylakoids membranes (15 µg ml^{-1} Chl), isolated from control plants and incubated with 15,7 µM Cu and 44,5 µM Cd in the measuring solution. The activity was measured in the presence of 0.4 mM PPBQ as artificial electron acceptor in the Hill reaction H_20 PPBQ. Values represent means ± se (n=5**)** **.** Spe: *S. pedicelata*, Sb: *S. babylonica*, Se: *S. eleagnos*, Spu: *S. purpurea*, Sa: *S. atrocinerea* Values followed by the same letter are not significantly different according to Duncan's test ($p<0.05$).

that much higher Cd^{2+} (mM range) were needed for inhibition (Faller et al., 1999); whereas, the sensitivity of chloroplast to toxic Cu concentrations differ between species. The most sensitive and tolerant ones were *S. eleagnos* and *S. purpurea,* respectively. The *targets* on PSII of both Cu and Cd, described in the literature, are similar (see review by Krupa and Baszynski, 1995). The difference between the behavior in chloroplasts of the two metals could be due to specific defense mechanisms generated in the chloroplast against the toxicity.

On the other hand, the effect of both Cu and Cd on the PSII activity "*in vitro*" was not correlated with those observed on growth *"in vivo"*. The experiments *in vitro* could give valuable information about the molecular mechanism of action from a metal; however, they do not reflect the situation in the whole plant (Krupa and Baszynski, 1995). In experiments *in vivo* the metal concentration inside the chloroplast from plants growing at toxic metal concentrations is often much lower than that used for *in vitro* experiments. In addition, we have to consider additional *targets* for the heavy metal action *in vivo*. Therefore, extrapolation of results obtained *in vitro* may lead to misinterpretation of effect *in vivo* (Van Assche and Clijisters, 1990).

Summarizing, this study shows the usefulness of the calculation of TI based on aerial growth for metal tolerance test of woody plants. In addition, the measurement of photosynthetic parameters indicates that metal-induced disturbances on growth could not be even partially attributed to changes on Chl content.

Cu-tolerant (*S. pedicelata*) and Cd-tolerant (*S. purpurea*) species may be effective in phytoremediation. Nevertheless, further studies on physiological responses to metal and accumulation are necessary to confirm feasibility of the phytoremediation technology using these species.

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