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Copper toxicity and sulfur metabolism in Chinese cabbage are affected by UV radiation

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ABSTRACT

Biomass production, dry matter content, specific leaf area and pigment content of Chinese cabbage were all quite similar, when plants were grown in the absence or presence of UV-A + B (2.2 mW cm^{-2}). Elevated Cu^{2+} concentrations (2–10 μM) in the root environment and UV radiation had negative synergistic effects for Chinese cabbage and resulted in a more rapid and stronger decrease in plant biomass production and pigment content. The quantum yield of photosystem II photochemistry (F_v/F_m) was only decreased at ≥5 μM Cu^{2+} in the presence of UV radiation, when leaf tissue started to become necrotic. The enhanced Cu toxicity in the presence of UV was largely due to a UV-induced enhanced accumulation of Cu in both roots and shoots. An enhanced Cu content strongly affected the uptake and assimilation of sulfur in plants. The total sulfur content of the root increased at ≥2 μM Cu^{2+} in presence of UV and at 10 μM Cu^{2+} in absence of UV and that of the shoot increased at ≥2 μM Cu^{2+} in presence of UV and at ≥5 μM Cu^{2+} in absence of UV. In the shoot it could be attributed mainly to an increase in sulfate content. Moreover, there was a strong increase in the water-soluble non-protein thiol content upon Cu^{2+} exposure in the root and, to a lesser extent in the shoot, both in the presence and absence of UV. The regulation of the uptake of sulfate responded to the occurrence of Cu toxicity directly, since it was more rapidly affected in the presence than in the absence of UV radiation. For instance, the expression and activity of the high affinity sulfate transporter, Sultr1;2, were enhanced at ≥2 μM in the presence of UV, and at ≥5 μM Cu^{2+} in the absence of UV. In the shoot, the expression of the vacuolar sulfate transporter, Sultr4;1, was upregulated at ≥5 μM Cu^{2+} in the presence and absence of UV whilst the expression of a second vacuolar sulfate transporter, Sultr4;2, was upregulated at 10 μM Cu^{2+} in the presence of UV. It is suggested that high Cu tissue levels may interfere/react with the signal compounds involved in the regulation of expression and activity of sulfate transporters. The expression of adenosine 5′-phosphosulfate reductase in the root was hardly affected and was slightly down-regulated at 2 μM in the presence of UV and at 10 μM in the absence of UV. The expression and activity of sulfate transporters were enhanced upon exposure at elevated Cu^{2+} concentrations; this may not be simply due to a greater sulfur demand at higher Cu levels, but more likely is the consequence of Cu toxicity, since it occurred more rapidly in the presence compared to the absence of UV.

1. Introduction

Copper levels in agricultural soil may be strongly enhanced as a consequence of anthropogenic activities through the application of organic fertilizers, use of sewage sludge as a fertilizer, application of sewage water for crop production and the use of Cu-containing fungicides (Dach and Starmans, 2005; Zhou et al., 2005; Yruela, 2005, 2009).

Copper (Cu) is an essential redox-active transition metal for normal plant growth and development and a cofactor in many metalloproteins, but elevated Cu concentrations in the root environment (≥5 μM) may rapidly become phytotoxic (Kopsell and Kopsell, 2007; Burkhead et al., 2009; Yruela, 2005, 2009; Shahbaz et al., 2010). Exposure of plants to elevated Cu concentrations generally results in leaf chlorosis, a loss of photosynthetic activity and in a reduced plant biomass production (Kopsell and Kopsell, 2007; Burkhead et al., 2009; Yruela, 2005, 2009; Shahbaz et al., 2010). However, leaf chlorosis in Chinese cabbage, which was first visible in young developing leaves, was not the consequence of pigment degradation, but probably due

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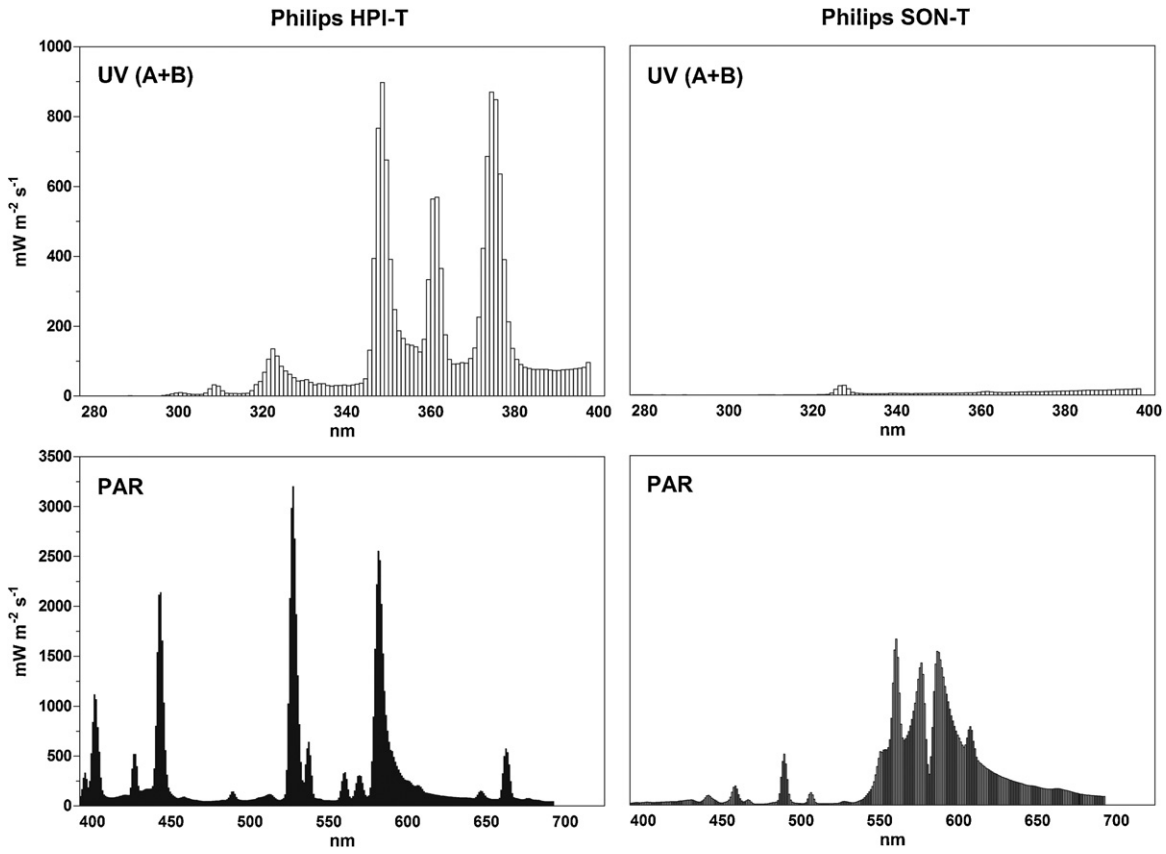

Fig. 1. Spectrum of light at plant height from Philips HPI-T and Philips SON-T lamps. UV (A + B), ultraviolet irradiation; PAR, photosynthetically active radiation.

to hindered chloroplast development at high Cu^{2+} concentrations (Shahbaz et al., 2010).

Elevated Cu concentrations may affect sulfur metabolism, since Cu is an activator of phytochelatins synthesis, which together with glutathione (or even free cysteine) may be involved in Cu binding and its detoxification (Sirko and Gotor, 2007). Exposure of Chinese cabbage to elevated Cu^{2+} concentrations in the root environment resulted in increase in water-soluble non-protein thiol content of the root and to a lesser extent of the shoot of Chinese cabbage, however, this increase could only be partially attributed to a Cu-induced accumulation of the phytochelatins (Shahbaz et al., 2010). The uptake of sulfate and its metabolism are controlled by the plant sulfur demand for growth (Hawkesford and De Kok, 2006; De Kok et al., 2011). Elevated Cu^{2+} concentrations affected the uptake, distribution and assimilation of sulfate in Chinese cabbage and both the expression and activity of sulfate transporters were enhanced at $\geq$2 μM Cu^{2+} (Shahbaz et al., 2010). However, it was unclear to what extent changes in sulfate uptake and metabolism upon Cu^{2+} exposure were the consequence of a higher sulfur demand in order to detoxify the excess Cu, or the result of a direct interference of Cu with the signal transduction pathway involved in sulfur metabolism (Shahbaz et al., 2010).

It has been predicted that there will be an increase in UV radiation on the earth surface due to ozone depletion in the stratosphere and reduction of aerosols and clouds (McKenzie et al., 2007). UV-B (280–320 nm) is the most harmful part of the UV spectrum for plants reaching the surface of the earth. UV-B absorption by foliage may result in the formation of reactive oxygen species, especially in chloroplasts (Tausz, 2001). Consequently, high UV-B levels may alter thylakoid integrity, induce pigment degradation and decrease chlorophyll *a*/*b* ratio, Rubisco activity and stomatal conductivity (Jansen et al., 1998; Larsson et al., 1998) and substantially affect plant performance. The level of pigment degradation was reported to be dependent on the length and intensity of the UV-B radiation (Kakani et al., 2003). Plant species differ in their ability to tolerate UV-B radiation and pigment degradation occurred more rapidly in dicotyledons than in monocotyledons (Kakani et al., 2003).

Cu has the potential to accelerate the formation of reactive oxygen species in plant tissues (Pinto et al., 2003) and the high light intensities which enhanced the toxicity of Cu to chloroplast functioning was presumably due to an enhanced production of hydroxyl radicals (Yruela et al., 1996; Yruela, 2009). The impact of Cu exposure and UV radiation on plants may be synergistic. For instance, both growth and chlorophyll content were reduced to a greater extent when the aquatic plant *Lemna gibba* was simultaneously exposed to Cu and UV radiation (Babu et al., 2003). Moreover, Cu toxicity was increased upon exposure to high light intensities and/or UV radiation for chloroplasts (Pätsikkä et al., 1998), green alga (West et al., 2003; Lupi et al., 1998), cyanobacteria (Rai et al., 1998; Gouvêa et al., 2008) and for duckweed (Babu et al., 2003), with symptoms indicative of synergistic oxidative damage.

The interactive effects of Cu and UV radiation have only been studied in cyanobacteria and in aquatic plant species. There is little known about the combined effects of elevated Cu levels and light quality (*viz.* UV radiation) in plants. The present study was undertaken to investigate (i) the impact of light quality (UV radiation) on copper toxicity, and (ii) the interaction of Cu toxicity with the regulation of the uptake and metabolism of sulfate in Chinese cabbage (*Brassica pekinensis*).

2. Materials and methods

2.1. Plant growth conditions

10-day-old seedlings were transferred to an aerated 25% Hoagland nutrient solution, pH 5.9–6.0, consisting of 1.25 mM