Copper toxicity and sulfur metabolism in Chinese cabbage

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UV radiation increases the toxicity and impact of copper on sulfur metabolism in Chinese cabbage
Abstract

Biomass production, dry matter content, specific leaf area and pigment content of Chinese cabbage were all quite similar whether plants were grown with HPI-T (2.2 mW cm\(^{-2}\), UV-A+B) or SON-T (0 mW cm\(^{-2}\), UV-A+B) lamps as a light source. Exposure of plants to enhanced Cu\(^{2+}\) concentrations (2–10 μM) resulted in a more rapid and stronger decrease in plant biomass production and pigment content in presence of UV radiation (HPI-T) compared to the absence of UV radiation (SON-T). The \(F_v/F_m\) ratio was only decreased at ≥ 5 μM Cu\(^{2+}\) in the presence of UV radiation, when leaf tissue started to become necrotic. The content of Cu in both root and shoot of Chinese cabbage was strongly affected by the level of UV radiation, both in absence and presence of enhanced Cu\(^{2+}\) concentrations in the root environment. The observed enhanced Cu toxicity in presence of UV is probably due largely to a higher accumulation of Cu in both root and shoot. The total sulfur content of the shoot was increased at ≥ 2 μM Cu\(^{2+}\) in presence of UV and at ≥ 5 μM Cu\(^{2+}\) in absence of UV, which was mainly attributed to an increase in sulfate content. Moreover, there was a strong increase in water-soluble non-protein thiol content upon Cu\(^{2+}\) exposure in the root and, to a lesser extent in the shoot, both in presence and absence of UV. The expression and activity of the high affinity sulfate transporter, Sultr1;2, was enhanced at ≥ 2 μM in presence of UV, and at ≥ 5 μM Cu\(^{2+}\) in absence of UV. Furthermore, in the shoot, the expression of vacuolar sulfate transporter, Sultr4;1, was upregulated at ≥ 5 μM Cu\(^{2+}\) in presence and absence of UV, whilst the expression of second vacuolar sulfate transporter, Sultr4;2, was upregulated at 10 μM Cu\(^{2+}\) in presence of UV. The expression of APS reductase in the root was hardly affected and it was slightly downregulated at 2 μM in presence of UV and at 10 μM, in absence of UV. The expression and activity of sulfate transporters were enhanced upon exposure at enhanced Cu\(^{2+}\) concentrations; this may be due not only to a greater sulfur demand at higher Cu levels, but more likely was the consequence of Cu toxicity, since it occurred more rapidly in presence of compared to the absence of UV.
Introduction

Exposure of Chinese cabbage (*Brassica pekinensis*) to $\geq 5$ $\mu$M Cu$^{2+}$ resulted in leaf chlorosis and a decrease in plant biomass production and pigment content (Chapter 3). The decrease in pigment content was accompanied by a loss of photosynthetic capacity, however, expressed on chlorophyll basis, the rate of photosynthesis was hardly affected at levels up to 10 $\mu$M Cu$^{2+}$ (Chapter 3). Leaf chlorosis, which was first visible in young developing leaves, was probably not the consequence of pigment degradation, but due to a hindered chloroplast development at high Cu$^{2+}$ concentrations (Chapter 3). Enhanced Cu concentrations affected the uptake, distribution and assimilation of sulfate in Chinese cabbage. Both the expression and activity of the sulfate transporters were enhanced at approx. 2-5 $\mu$M Cu$^{2+}$. At $\geq 1$ $\mu$M Cu$^{2+}$ there was an increase in water-soluble non-protein thiol content of the root and to a lesser extent of the shoot of Chinese cabbage. This increase could only be partially attributed to a Cu-induced accumulation of the phytochelatins (Chapter 3). Cu$^{2+}$ exposure hardly affected the total sulfur content of the root of Chinese cabbage, whereas that of the shoot was increased at $\geq 1$ $\mu$M, the latter mainly due to an increase in sulfate content (Chapter 3). It remains to be resolved 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.

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 the foliage may result in the formation of reactive oxygen species, especially in the 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 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 potential also to accelerate the formation of reactive oxygen species in plant tissue (Pinto et al. 2003) and the high light intensities which may enhance the toxicity of Cu to chloroplasts 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 in 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 duck weed (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 aquatic plant species. There is little known about the combined effects of enhanced 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 Cu toxicity, and (ii) the interaction of Cu toxicity with the regulation of the uptake and metabolism of sulfate in Chinese cabbage (*Brassica pekinensis*).

**Plant growth conditions**

10-day-old seedlings were transferred to a 25% Hoagland nutrient solution, containing supplemental concentrations of 0, 2, 5 and 10 μM CuCl₂ in 13 l stainless steel containers (10 sets of plants per container, 3 plants per set) in a climate-controlled room for 7 days. Day and night temperatures were 25 and 20 °C (± 1 °C), respectively, and the relative humidity was 60-70%. The photoperiod was 14 h at a photon fluence rate of 500 ± 25 μmol m⁻² s⁻¹ (within the 400-700 nm range) at plant height. For the different light quality and UV-A+B radiation studies, the climate-controlled room was split into two parts by a non-transparent thermopore sheet. In one part light was supplied by Philips HPI-T lamps (400 W) and in the other part by
Philips SON-T lamps (600 W). HPI-T lamps had 2.2 mW cm\(^{-2}\) UV-A+B (within the 280-400 nm range) and 0.06 mW cm\(^{-2}\) UV-B radiation (within the 280-325 nm range), whereas SON-T lamps had almost no UV radiation, measured with a Digital Ultraviolet Radiometer model 5.0 and model SM 6.0, respectively (Solartech Inc. Fenton, MI 48430, USA). The spectrum of light from both the lamps is shown in figure 1. In experiments with UV filtered out, light was provided by HPI-T lamps and the UV (A+B) radiation was filtered out with a Perspex sheet (ISPA Plastics Groningen, The Netherlands).

![Image of the spectrum of light from Philips HPI-T and Philips SON-T lamps. UV (A+B), ultraviolet irradiation; PAR, photosynthetically active radiation.](image)

**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.
Results

Impact of Cu and light quality on growth, dry matter content, pigment content and Cu content

Irrespective of whether Chinese cabbage was grown with HPI-T and SON-T lamps as the light source, plant biomass production, dry matter content, shoot pigment content and the specific leaf area were all quite similar, however, shoot to root ratio was slightly lower with HPI-T (Fig. 2 and 3). However, if plants were exposed to enhanced Cu\(^{2+}\) concentrations in the nutrition solution, biomass production was more rapidly affected with the HPI-T compared to SON-T light source. With HPI-T, Cu\(^{2+}\) exposure resulted in a significant decrease in plant biomass production at ≥ 2 µM (up to 70% at 10 µM) accompanied by a decrease in specific leaf area, whereas the shoot to root ratio was only slightly increased (Fig. 2). However, with SON-T, plant biomass production was only slightly decreased at ≥ 5 µM Cu\(^{2+}\) (up to 34% at 10 µM), whereas shoot to root ratio only significantly increased at 10 µM (Fig. 2). At ≥ 5 µM Cu\(^{2+}\) there was an increase in dry matter content in both root and shoot with HPI-T, whereas it was hardly affected at all with SON-T (Fig. 2).

The pigment content started to decrease with both HPI-T and SON-T at ≥ 2 µM Cu\(^{2+}\), but similarly to the observations on biomass production, the decrease occurred more rapidly with HPI-T compared to SON-T (Fig. 3). For example, at 2 µM Cu\(^{2+}\), pigment content had decreased to 55 and 21% with HPI-T and SON-T, respectively (Fig. 3). If plants were exposed to ≥ 5 µM Cu\(^{2+}\) and HPI-T, not only the pigment content was decreased, but there was also a change in pigment composition. Here, chlorophyll \(a\) content decreased significantly faster than that of chlorophyll \(b\) and carotenoids, resulting in a significantly decreased chlorophyll \(a/b\) and chlorophyll/carotenoid ratio (Fig. 3). With SON-T, the chlorophyll \(a/b\) and chlorophyll/carotenoid ratios were only slightly decreased at ≥ 5 µM Cu\(^{2+}\) (Fig. 3). The quantum yield of photosystem II photochemistry (\(F_v/F_m\)) only decreased in Chinese cabbage leaves upon exposure to ≥ 5 µM Cu\(^{2+}\) with HPI-T whereas, with SON-T it was not affected (Fig. 3).
Fig. 2. Impact of Cu^{2+} and light quality on biomass production, dry matter content and specific leaf area of Chinese cabbage. 10-day-old seedlings of Chinese cabbage were grown to 25% Hoagland solution containing 0, 2, 5 and 10 µM CuCl$_2$ for 7 days. Plants were grown with HPI-T (black bars) and SON-T lamps as a light source (grey bars). The initial shoot and root weight was 0.045 ± 0.004 and 0.009 ± 0.001 g fresh weight, respectively. The data on biomass production (g FW), dry matter content (DMC; %) and shoot/root ratio represent the mean of 6 experiments with 9 measurements with 3 plants in each (± SD), and specific leaf area (SLA; cm$^{-2}$ g$^{-1}$ FW) represents the mean of 6 measurements with 3 plants in each (± SD). Different letters indicate significant differences between treatments (p < 0.01, Student’s t-test).
Fig. 3. Impact of Cu\textsuperscript{2+} and light quality on pigment content, pigment composition and chlorophyll \textit{a} fluorescence. For experimental details see legends Fig. 2. Plants were grown with HPI-T (black bars) and SON-T lamps as a light source (grey bars). The pigment content (mg g\textsuperscript{-1} FW) represents the mean of 2 experiments with 3 measurements in each (± SD) and chlorophyll \textit{a} fluorescence (\(F_\text{v}/F_\text{m}\) ratio) the mean of 9 measurements (± SD). Different letters indicate significant differences between treatments (\(p < 0.01\), Student’s t-test).

Chinese cabbage grown with HPI-T had a 40% higher Cu content in both root and shoot compared to plants grown with SON-T (Fig. 4). With both HPI-T and SON-T, the Cu content of both root and shoot increased with the Cu\textsuperscript{2+} concentration in the nutrient solution, however, plants grown with HPI-T accumulated more Cu than with SON-T upon exposure to Cu\textsuperscript{2+} (Fig. 4) For example, at 2 µM Cu\textsuperscript{2+}, the Cu content in root of HPI-T and SON-T was 4.18 and 1.54 μmol g\textsuperscript{-1} DW, respectively. The accumulation of Cu upon exposure of plants to high Cu concentrations in the nutrient solution differed strongly between root and shoot, since the excessive Cu taken up by the root appeared to be rather immobile. For instance, at 10 µM Cu\textsuperscript{2+} the Cu content of root increased 11-fold after 7 days of exposure, whereas in
the shoot it was only increased up to 1.3-fold with HPI-T. With SON-T the content of Cu increased in root up to 14-fold and in the shoot, up to 2.8-fold with 10 µM Cu\(^{2+}\) (Fig. 4).

In order to determine the significance of UV radiation on the impact of enhanced Cu concentration on Chinese cabbage, plants were grown under conditions where the UV-A+B radiation of the HPI-T lamps was filtered out by a UV-absorbing Perspex sheet (HPI-T*). Biomass production and pigment content of Chinese cabbage grown with HPI-T and HPI-T* were quite similar (Fig. 5). However, the observed decrease in plant biomass production, pigment content and chlorophyll fluorescence at ≥ 2 µM Cu\(^{2+}\) with HPI-T was largely diminished with HPI-T*, demonstrating that UV-A+B radiation strongly enhanced the phytotoxicity of enhanced Cu\(^{2+}\) concentrations (Fig. 5).

**Fig. 4.** Impact of Cu\(^{2+}\) and light quality on Cu content. For experimental details see legends Fig. 2. Plants were grown with HPI-T (black bars) and SON-T lamps as a light source (grey bars). The Cu content (µmol g\(^{-1}\) DW) represents the mean of 3 measurements with 9 plants in each (± SD). Different letters indicate significant differences between treatments (p < 0.01, Student’s t-test).

**Impact of Cu and light quality on nitrogen and sulfur metabolite content**

The nitrate, amino acid and sulfur metabolite contents of Chinese cabbage were quite similar for both HPI-T and SON-T –grown plants. Upon Cu\(^{2+}\) exposure, the nitrate content was slightly decreased in the
root at $\geq 2 \mu M$ and at $10 \mu M Cu^{2+}$ in the shoot with both HPI-T and SON-T (Fig. 6 and 7). There was a strong increase in amino acid content of the shoot upon Cu$^{2+}$ exposure at $\geq 2 \mu M$ and to a lesser extent in the root with HPI-T, whereas it was hardly affected with SON-T (Fig. 6). At 2 $\mu M Cu^{2+}$ there was a substantial increase in total sulfur content of the shoot and at $\geq 5 \mu M$ in the root, with HPI-T. However, with SON-T, Cu$^{2+}$ concentrations of $\geq 5 \mu M$ had a substantial effect on total sulfur content of the shoot whereas the content of the root was only increased at 10 $\mu M Cu^{2+}$. The increase in total sulfur content upon Cu$^{2+}$ exposure could for the greater part be attributed to enhance sulfate content (Fig. 7). Moreover, upon Cu$^{2+}$ exposure at $\geq 2 \mu M$, there was a strong increase in water-soluble non-protein thiol content in the root and to a lesser extent in the shoot (at $\geq 5 \mu M Cu^{2+}$) for both HPI-T and SON-T (Fig. 7).

Impact of Cu and light quality on sulfate uptake capacity and expression of sulfate transporters and APS reductase

Both the expression of the high affinity sulfate transporter Sultr1;2 and the sulfate uptake capacity of the root of Chinese cabbage were slightly higher with SON-T compared to HPI-T illumination (Fig. 8 and 9). The expression of the Group 2 sulfate transporter Sultr2;2, which is involved in vascular transport, was also slightly more expressed with SON-T compared to HPI-T –grown plants, in root (Fig. 9). The expression of the Group 4 sulfate transporter Sultr4;1, which is involved in vacuolar efflux of sulfate, was quite similar for both HPI-T and SON-T illumination, but in the shoot expression was 40% lower with SON-T (Fig. 9).

Upon Cu$^{2+}$ exposure of HPI-T grown plants, there was a strong increase in sulfate uptake capacity at $\geq 2 \mu M$ (about 2-fold), whereas it was slightly decreased at 10 $\mu M Cu^{2+}$ (Fig. 8). With SON-T illumination, sulfate uptake capacity was increased at $\geq 5 \mu M Cu^{2+}$ (Fig. 8). Both for HPI-T and SON-T illumination, the increase in sulfate uptake capacity upon Cu$^{2+}$ exposure was accompanied with an enhanced expression of Sultr1;2 in the root, whereas Sultr1;1 was hardly expressed (Fig. 8 and 9). With HPI-T illumination, the expression of Sultr1;2 in roots was increased by 159 and 77% at 2 and $5 \mu M Cu^{2+}$, respectively, whereas with SON-T illumination, expression was increased by 40% at $\geq 5 \mu M Cu^{2+}$ (Fig. 9). Upon Cu$^{2+}$
exposure with HPI-T illumination, the expression of Sultr2;2 was slightly upregulated in root at 2 µM Cu²⁺, however with SON-T there was no response (Fig. 9). Upon Cu²⁺ exposure, the expression of Sultr4;1 was hardly affected in the root under both HPI-T and SON-T illumination. However, for HPI-T illumination, the expression of Sultr4;1 was increased by 53 and 166% at 5 and 10 µM Cu²⁺ respectively, and for SON-T illumination, its expression was increased by 50% at 10 µM Cu²⁺ in shoot (Fig. 9). Sultr4;2 was slightly expressed in root with SON-T illumination, but upon Cu²⁺ exposure expression was strongly decreased. However, in shoot, the expression of Sultr4;2 was increased 1.4-fold and 3.6-fold at 5 and 10 µM Cu²⁺, respectively, under HPI-T illumination, but was hardly expressed under SON-T illumination (Fig. 9). The expression of APS reductase in both root and shoot was quite similar for both HPI-T and SON-T grown plants, and expression was slightly enhanced in root upon Cu²⁺ exposure. However, in shoot the expression of APS reductase was slightly decreased at 2 µM Cu²⁺ under HPI-T, and at 10 µM Cu²⁺ under SON-T illumination (Fig. 9).

Discussion

A simultaneous exposure of plants to enhanced Cu levels and UV radiation may result in synergistic negative effects on plant growth and functioning (Lupi et al. 1998; Babu et al. 2003). From the current study it was evident that growth and metabolite content of Chinese cabbage was hardly affected upon illumination with HPI-T and SON-T lamps as light sources, despite the large differences in light spectrum (viz. UV level). However, if Chinese cabbage was exposed to enhanced Cu²⁺ concentrations in the root environment, toxic effects occurred more rapidly with HPI-T compared to SON-T illumination. The differences in the development of toxic effects of Cu²⁺ at different light conditions, could be solely ascribed to differences in the level of UV radiation upon illumination with HPI-T (UV-A+B) and SON-T (no UV-A+B) lamps. The negative effects of enhanced Cu²⁺ concentrations on biomass production, pigment content and the Fv/Fm ratio in Chinese cabbage was strongly decreased in absence of UV-A+B (SON-T lamps) and when the UV-A+B of the HPI-T lamps was filtered out. Moreover, with HPI-T lamps, enhanced Cu²⁺ concentrations also
resulted in a change in leaf morphology of Chinese cabbage, illustrated by a decrease in specific leaf area (SLA).

**Fig. 5.** Impact of Cu\(^{2+}\) and UV radiation on biomass production, pigment content and chlorophyll a fluorescence. 10-day-old seedlings of Chinese cabbage were grown on 25% Hoagland solution containing 0, 2, and 5 µM CuCl\(_2\) for 7 days. Plants were grown with HPI-T (black bars) and UV radiation filtered out from HPI-T* lamps by a Perspex sheet (white bars). The initial shoot and root weight was 0.050 ± 0.002 and 0.008 ± 0.001 g fresh weight, respectively. The data on biomass production (g FW) and shoot/root ratio represent the mean of 9 measurements with 3 plants in each (± SD). The pigment content (mg g\(^{-1}\) FW) represents the mean of 2 experiments with 3 measurements in each (± SD) and chlorophyll a fluorescence (\(F_v/F_m\) ratio) the mean of 9 measurements (± SD). Different letters indicate significant differences between treatments (\(p < 0.01\), Student’s t-test).
Fig. 6. Impact of Cu$^{2+}$ and light quality on the content of nitrate and amino acids. For experimental details see legends Fig. 2. Plants were grown with HPI-T (black bars) and SON-T lamps as a light source (grey bars). The nitrate and amino acid content ($\mu$mol g$^{-1}$ FW) represent the mean of 2 experiments with 3 measurements in each ($\pm$ SD). Different letters indicate significant differences between treatments (p < 0.01, Student’s t-test).

The content of Cu in both root and shoot of Chinese cabbage was strongly affected by the level of UV, both in absence and presence of enhanced Cu$^{2+}$ concentrations in the root environment. In absence of enhanced Cu$^{2+}$ concentrations, Chinese cabbage grown with HPI-T illumination had a 40% higher Cu content in root and shoot compared to SON-T grown plants. Moreover, the accumulation of Cu at enhanced Cu$^{2+}$ concentrations was always substantially higher in both root and shoot of Chinese cabbage grown with HPI-T rather than SON-T illumination (in root even up 2.5-fold). The observation that Cu was more toxic in presence than in absence of UV is probably largely due to the observed differences in accumulation of Cu in root and shoot of Chinese cabbage. Similar to previous observations (Chapter
3), the excessive Cu taken up by the root was rather immobile and on a whole plant basis not more than 20% was transferred to the shoot.

![Graph showing the impact of Cu²⁺ and light quality on the content of total sulfur, sulfate and water-soluble non-protein thiols.](image)

**Fig. 7.** Impact of Cu²⁺ and light quality on the content of total sulfur, sulfate and water-soluble non-protein thiols. For experimental details see legends Fig. 2. Plants were grown with HPI-T (black bars) and SON-T lamps as a light source (grey bars). The sulfate and water-soluble non-protein thiol content (µmol g⁻¹ FW) represent the mean of 2 experiments with 3 measurements in each (± SD). Total sulfur content (µmol g⁻¹ DW) represents the mean of 3 measurements (± SD). Different letters indicate significant differences between treatments (p < 0.01, Student’s t-test).
Enhanced Cu levels as well as UV radiation have the potential to induce oxidative stress by the formation of reactive oxygen species (Lupi et al. 1998; Tausz 2001; Babu et al. 2003), which might also have contributed the development of leaf necrosis at ≥ 5 µM Cu$^{2+}$ with HPI-T illumination. Exposure of *Lemna gibba* to UV radiation and enhanced Cu concentrations resulted in an enhanced activity of Cu/ZnSOD (Babu et al. 2003). Cu/ZnSOD performs a significant role in the detoxification of reactive oxygen species and expression in the cytosol and chloroplasts was upregulated at high light and enhanced Cu levels (Tausz 2001; Pilon et al. 2011). This enzyme is the most abundant Cu protein along with plastocyanin in green plants (Yruela 2005, 2009, Cohu and Pilon 2007). To what extent the observed enhancement of Cu content in Chinese cabbage with HPI-T illumination was the consequence of UV radiation-induced enhanced Cu uptake for synthesis of Cu/ZnSOD is not known.

Similar to previous observations (Chapter 3), the decrease in pigment content at enhanced Cu$^{2+}$ concentrations in the root environment with HPI-T (and UV-filtered out HPI-T) and SON-T illumination was not accompanied with a change quantum yield of photosystem II photochemistry ($F_v/F_m$ ratio) indicating that the remaining chloroplasts were functional. Only at ≥ 5 µM Cu$^{2+}$ upon HPI-T illumination, where leaf tissue started to become necrotic, there was a decrease in $F_v/F_m$ ratio, confirming higher toxicity of Cu in presence of UV.

Exposure of Chinese cabbage to enhanced Cu concentrations substantially affected the content and distribution of sulfur compounds in the root and shoot of Chinese cabbage (Chapter 3). There was a strong accumulation of water-soluble non-protein thiols in the root and, to a lesser extent in the shoot. However, increase in thiol content in Chinese cabbage could only partially be ascribed to a Cu-induced synthesis of phytochelatins (Chapter 3). There was enhanced total sulfur content in both root and shoot which could be attributed to sulfate accumulation. The enhanced sulfur content upon Cu$^{2+}$ exposure was most likely the consequence of upregulation of the expression and activity of sulfate transporters in the root of Chinese cabbage. Expression and activity of sulfate transporters in Chinese cabbage were slightly affected by HPI-T and SON-T illumination, however, expression was strongly increased at enhanced Cu$^{2+}$ concentrations. Expression and activity of Sultr1;2 in the root of
Chinese cabbage were slightly enhanced with SON-T compared to HPI-T illumination, which was most likely due to a slightly higher shoot biomass production. It was evident that the rate of sulfate uptake capacity by the root was determined by the sink capacity of the shoot (Koralewska et al. 2009b).

Fig. 8. Impact of Cu\(^{2+}\) and light quality on sulfate uptake capacity. For experimental details see legends Fig. 2. Plants were grown with HPI-T (black bars) and SON-T lamps as a light source (grey bars). Data on sulfate uptake capacity (\(\mu\text{mol g}^{-1} \text{FW root h}^{-1}\)) represent the mean of 3 measurements with 3 plants in each (\(\pm\) SD). Different letters indicate significant differences between treatments (\(p < 0.01\), Student’s t-test).

The uptake, distribution and assimilation of sulfur at a whole plant level are controlled by the plant sulfur status and the sulfur demand to maintain the plant growth (Hawkesford and De Kok 2006; Rouached et al. 2009). It has been proposed that both low and high-affinity sulfate transporters may play key roles in sulfate uptake, translocation and distribution in higher plants (Buchner et al. 2004; Hawkesford and De Kok 2006). However, the mechanism by which Cu influences the sulfur status of the plants and the altered gene expression of both sulfate transporters and APS reductase is not clear. It may be that Cu itself interferes/reacts with the regulatory
signal compounds involved in the regulation of the sulfate transporters (Chapter 3).

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<tr>
<th>Light source</th>
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<th>Shoot</th>
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<tr>
<td>Cu²⁺μM</td>
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Fig. 9. Impact of Cu²⁺ and light quality on mRNA abundance of sulfate transporters (Sultr) and APS reductase (APR; Northern-blot analysis) in the root and shoot of Chinese cabbage. Equal RNA loading was determined by ethidium bromide staining of gels (shown in the bottom panels). For experimental details see legends Fig. 2. A representative set of data from two independent experiments is given.

The expression of the constitutively expressed sulfate transporter, Sultr4;1, and the wholly inducible sulfate transporter, Sultr4;2, was upregulated in the shoot upon Cu²⁺ exposure at enhanced levels (≥ 5 μM); generally these sulfate transporters only upregulated upon sulfate deficiency (Buchner et al. 2004; Parmar et al. 2007; Koralewska et al. 2009a,b; Stuiver et al. 2009). The expression and activity of APS reductase is generally downregulated at enhanced thiol levels (Westerman et al. 2001; Durenkamp et al. 2007; Koralewska et al. 2008). However, similarly to previous observations, the strongly enhanced thiol levels in the root and to a lesser extent in the shoot did not result in downregulation of the expression of APS reductase (Chapter 3). Whereas, the expression of APS reductase was slightly
downregulated at 2 and 10 µM Cu$^{2+}$ with HPI-T and with SON-T illumination respectively, which may be due to an enhanced sulfate content of the shoot. It may be that the sulfate content rather than the thiol concentration was of greater significance in the regulation of the expression of APS reductase in the shoot.

From the present data it was evident that the upregulated expression and activity of sulfate transporters upon Cu$^{2+}$ exposure at enhanced levels was likely not due to a higher sulfur need at higher tissue Cu contents, since plants took up more sulfate than was metabolized, resulting in an enhanced sulfate content of the shoot (Chapter 3). Furthermore, the water-soluble non-protein thiols which are presumed to be involved in binding/chelating excessive Cu in plant tissue, accumulated at quite similar levels with HPI-T and SON-T illumination irrespective of the development of Cu toxicity. However, the observed enhancement in expression and activity of sulfate transporters, total sulfur content and sulfate content upon Cu$^{2+}$ exposure, occurred more rapidly in presence of UV radiation (HPI-T) than in absence of UV radiation (SON-T). Therefore, increases in sulfate uptake, thiol content and total sulfur content may not only be due to a higher sulfur need, but may be specifically a response to the occurrence of Cu toxicity, as the increases appear more rapidly in presence of UV radiation.

**Conclusions**

Enhanced Cu$^{2+}$ concentrations in the root environment and UV radiation had negative synergistic effects in Chinese cabbage. The negative effects of enhanced Cu$^{2+}$ concentrations on biomass production, pigment content and the $F_v/F_m$ ratio in Chinese cabbage were strongly increased in presence of UV illumination. Enhanced Cu toxicity in presence of UV was largely due to a UV-induced enhanced accumulation of Cu content in both root and shoot of Chinese cabbage. Enhanced Cu content in the root affected the regulation of the uptake and distribution of sulfate, which was due not only to higher sulfur need for its detoxification. It is suggested that the regulation also responded to the occurrence of Cu toxicity directly, since this occurred more rapidly in presence of UV radiation compared to the absence of UV radiation. Furthermore, it is suggested that the
enhanced Cu content in the roots interferes/reacts with the signal compounds involved in the regulation of expression and activity of sulfate transporters.