Environmental and Experimental Botany 79 (2012) 58–65

Contents lists available at SciVerse ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Differences in copper accumulation and copper stress between eight populations of *Haumaniastrum katangense*

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ARTICLE INFO

Article history:
Received 28 July 2011
Received in revised form
13 November 2011
Accepted 13 December 2011

Keywords:
Chlorophyll fluorescence kinetics
Copper tolerance
Copper toxicity
Population comparison
Iron uptake
Metal accumulation

ABSTRACT

Haumaniastrum katangense is well known as the "copper flower" of the Katangan Copper Belt. Few studies, however, are available on the physiology of this remarkable plant, including questions like stress responses and population-dependent differences. In the current study, we compared the response to copper for eight populations of this species in terms of copper accumulation, copper resistance, and various physiological parameters that might change under copper toxicity stress (biophysics of photosynthesis, growth, chlorophylls and carotenoids). Among six populations growing well under experimental conditions, three were found to be copper sensitive in terms of a strong inhibition of growth by 10 μM copper, while the other three were rather resistant. As the most prominent copper tolerance associated difference, copper resistant populations (as judged by their growth, photosynthetic activity and pigmentation) showed a decrease of iron accumulation in response to increased copper supply, while copper sensitive populations increased their Fe accumulation in response under these conditions. Copper sensitive populations showed the expected loss of pigments under copper toxicity stress, while two of the three copper tolerant populations even showed an enhancement of chlorophylls and violaxanthin in response to toxic copper. Also for other pigments population-specific differences in copper response were found, but they did not correlate with copper tolerance. Photosynthesis biophysics was affected by copper stress like in other species, no clearly tolerance/population-specific differences were found.

Abbreviations

- F_0 minimal fluorescence yield of a dark adapted sample, fluorescence in non-actinic measuring light
- F_m maximum fluorescence yield of a dark-adapted sample after supersaturating irradiation pulse
- F'_m maximum fluorescence yield of a light-adapted sample after supersaturating irradiation pulse
- F_v variable fluorescence; $F_v = F_m - F_0$
- $F_v/F_m = (F_m - F_0)/F_m$ maximal dark-adapted quantum yield of PSII photochemistry
- $\Phi_{PSII} = \Phi_e = (F'_m - F'_t)/F'_m$ the light-acclimated efficiency of PSII (Genty et al., 1989). In the current manuscript the use of this parameter is extended to the relaxation period after the end of actinic light to analyse the return of the system to its dark-acclimated state as measured by F_v/F_m.
- NPQ non-photochemical quenching, in this manuscript used as an acronym for the name of this phenomenon. In this manuscript, we measure non-photochemical quenching as $qCN = (F_m - F'_m)/F_m$ = "complete non-photochemical quenching of Chl fluorescence", i.e. with normalisation to F_m.

1. Introduction

Copper is an essential micronutrient for growth and development of animals (incl. humans) and plants at its optimal concentrations, but the excess of copper in organisms can lead to toxicity (reviewed e.g. by see Küpper and Kroneck, 2005; Burkhead et al., 2009). Such toxicity can originate from the use of copper in agriculture, e.g. as the "Bordeaux Mixture" for vine downy mildew

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doi:10.1016/j.envexpbot.2011.12.015

Table 1
Populations of *H. katangense* used for copper stress experiments.

No.	Site	Collection date	Longitude (E)	Latitude (S)	Altitude (m)	Site
2	Ruashi mine	1.vii.2006	27°32′30″	11°37′00″	1290	Copper mine on natural copper site (embankment)
20	Mangayabo field	1.vii.2006	27°36′20″	12°03′05″	1190	Occurrence of *H. katangense* on non-metalliferous site
22	Etoile mine	20.vi.2006	27°35′10″	11°38′00″	1261	Metalliferous mine site (steppe savannah)
23	Kamalondo railway	22.vi.2006	27°29′09″	11°41′01″	1207	Railway joining copper site to copper ore processing plant (anthropogenically copper-polluted site on ballast enriched with small pieces of rocks rich in malachite due to losses from copper ore transporting trains)
23a	Kamalondo "Cuivre Malant"	22.vi.2006	27°29′12″	11°41′01″	1199	Polluted site by local man activity (traditional copper rocks working place)
25	Kipushi	25.vi.2006	27°14′30″	11°46′05″	1329	Road verge near copper mining site (anthropogenically copper-polluted site), entry of shaft V
26	Karavia	26.vi.2006	27°25′05″	11°39′20″	1298	Ruderal site in the area of the Katangan Copper Bow (anthropogenically copper-polluted site, place of old copper smelting furnaces)
28	Kasombo mine	4.vii.2006	27°19′05″	11°40′15″	1267	Deserted old copper mine (embankment)
30	Luiswishi mine	30.vi.2006	27°25′45″	11°30′45″	1320	Copper mine on natural copper site

disease as well as in other pesticides. Runoff from agricultural fields can reach several micromolar copper (Gallagher et al., 2001; Ribolzi et al., 2002; Zhang et al., 2003; He et al., 2009), a concentration that is lethal for many aquatic plants (review by Küpper and Kroneck, 2005). Copper pollution can also result from industrial activities such as copper mining and waste deposition (Ke et al., 2007). As the result of a long term field application, copper residues can cause impacts on soil biota by reducing soil microbial biomass and eliminating earthworms in the orchard (Van-Zwieten et al., 2004). But the excess copper in soils also leads to growth reduction and may even be lethal for terrestrial plants (Rhoads et al., 1989; Flemming and Trevors, 1989).

The symptoms of copper toxicity are a series of physiological alterations which occur at the cellular and molecular level, resulting in functional changes that ultimately lead changes in morphology. For example, significant reductions of root calcium and iron contents, as well as extensive damage to root epidermal cells in maize plants were observed (Ouzounidou et al., 1995). Copper impairs cellular transport processes and changes biochemical metabolism dramatically (Hall, 2002). Already at low concentrations, Cu inhibits photosynthesis by the formation of Cu-chlorophylls, which are unsuitable for photosynthesis – the identified main target is PSII (Küpper et al., 1996, 1998, 2002; Mijovilovich et al., 2009; see Küpper et al., 2006 for a comprehensive review).

Therefore urgent need to remediate Cu contaminated soils. With the emerging technology of higher plants for phytoremediation, in order to clean up the Cu-contaminated soil and environments, investigations on the selection of suitable plant species and the understanding of biological mechanisms have been intensively conducted (for recent reviews see Lasat, 2000, 2002; Hall, 2002; Küpper and Kroneck, 2005). Phytoremediation of contaminated soil is a both cost-effective and environmental friendly method. Certain species have a high capability of Cu tolerance and/or accumulation, while most others are sensitive to Cu stress. Hyperaccumulators of heavy metals are plant species that actively accumulate metals in their shoots. A commonly used threshold for defining hyperaccumulators is an accumulation more than 100 times higher compared to non-accumulator plants. For Cu, this threshold would be around 1000 mg kg^{-1} (Lasat, 2002; Küpper et al., 2009a; Paton and Brooks, 1996).

Recent research identified the amphibious water plant *Crassula helmsii* as a new Cu accumulator for water environments. The shoots of the plant are able to accumulate Cu in a concentration beyond 9000 mg kg^{-1} in 0.6 mg kg^{-1} Cu^{2+} nutrient solution, but they are unsuitable for phytoremediation due to their growth characteristics (Küpper et al., 2009a).

Many plants of the genus *Haumaniastrum* grow in soils with exceedingly high contents of copper and cobalt. Some of them even grow only or mostly over Cu deposits; notably *Haumaniastrum katangense* is well known as the "copper flower" of the Katangan Copper Belt (called "la fleur du cuivre" by Duvigneaud, 1958). Those plants colonize mining sites and are widely distributed on the Cu-rich soils of Upper Katanga and western Zambia (Malaisse and Brooks, 1982; Leteinturier et al., 1999; Malaisse et al., 1999). These plants tolerate very high Cu levels, and an earlier study suggested that it may be a Cu hyperaccumulator (Paton and Brooks, 1996). But it remained unclear how much of the metal in these field-collected samples was actually taken up into the plant via the root or how much just became attached to the plants (incl. "uptake" into the stomata) as dust – such dust/aerosol "uptake" was shown to be potentially a large proportion (Faucon et al., 2007). In some studies, Cu levels in this species were clearly not in the hyperaccumulator range (e.g. Brooks, 1977; Chipeng et al., 2010). Further, for other hyperaccumulator species very strong differences in metal accumulation potential were found (as a classical case, see the Cd accumulation differences in *Thlaspi caerulescens* ecotypes, Lombi et al., 2000), so that the variation in Cu contents found for *H. katangense* could be soil-based or population-based.

H. katangense is presently known to occur in Angola, Dem. Rep. Congo, Tanzania and Zambia. In Tanzania, three districts are concerned, namely Ufipa, Nkansi and Sumbawanga districts; for these sites no heavy metal contents in soil are reported. In Zambia, *H. katangense* populations exist on Cu sites of the Copperbelt, and other places where no Cu rocks have been reported. The first group