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The effect of aluminium-stress and exogenous spermidine on chlorophyll degradation, glutathione reductase activity and the photosystem II D1 protein gene (*psbA*) transcript level in lichen *Xanthoria parietina*

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ABSTRACT

In this study, the effects of short-term aluminium toxicity and the application of spermidine on the lichen *Xanthoria parietina* were investigated at the physiological and transcriptional levels. Our results suggest that aluminium stress leads to physiological processes in a dose-dependent manner through differences in lipid peroxidation rate, chlorophyll content and glutathione reductase (EC 1.6.4.2) activity in aluminium and spermidine treated samples. The expression of the photosystem II D1 protein (*psbA*) gene was quantified using semi-quantitative RT-PCR. Increased glutathione reductase activity and *psbA* mRNA transcript levels were observed in the *X. parietina* thalli that were treated with spermidine before aluminium-stress. The results showed that the application of spermidine could mitigate aluminium-induced lipid peroxidation and chlorophyll degradation on lichen *X. parietina* thalli through an increase in *psbA* transcript levels and activity of glutathione reductase (GR) enzymes.

Introduction

Lichens can accumulate a large amount of metals because they have large surface areas and do not have cuticles or stoma in the thalli. Particles coming from the air through dry or wet deposition or soluble heavy metals are taken in by lichens *via* passive transport mechanisms, such as ion exchange and ion diffusion, or via active transport mechanisms (Nash III, 1996). Therefore, the accumulation, toxicity, and tolerance of metals in lichen thalli have been widely studied (Chettri et al., 1998; Garty et al., 1985; Sanita di Toppi et al., 2008; Unal et al., 2010; Pirintsos et al., 2004). Chlorophyll degradation and altered assimilation pigments of lichens in the field and in the laboratory were previously correlated with the presence of metals (Chettri et al., 1998). Currently, there are many studies on the different mechanisms of metal detoxification, including the production of lichen acids, phytochelatins, non-thiol compounds and antioxidant enzymes. Sanita di Toppi et al. (2008) showed that soluble antioxidants, such as glutathione and ascorbate, and antioxidant enzymes, such as superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), and peroxidases (POD), are involved during the entire process of metal detoxification and participate in metal-chelation (“first line”) and antioxidant protection (“second line”).

Polyamines, including putrescine (Put), spermidine (Spd), and spermine (Spm) are a class of biogenic amines with multiple *in vivo* effects on the cellular processes in most organisms (Bouchereau et al., 1999). Polyamines are important in protecting plants against abiotic stresses, such as potassium deficiency, osmotic shock, drought, metal stress and UV irradiation, because these compounds have roles in osmotic adjustment, maintenance of membrane stability and free-radical scavenging (Duan et al., 2009; Unal et al., 2008). In addition, an induction of polyamine biosynthesis has been shown to be a signal of stress tolerance in several systems. The data on polyamines in lichens indicate a correlation between polyamines and metal accumulation (Pirintsos et al., 2004), effects on nitrogen stress (Pirintsos et al., 2009) and a protective role against UV-A irradiation (Unal et al., 2008). Despite the large number of publications describing the effects of metal tolerance and toxicity on lichen physiology, the protective effects of polyamines under metal stress are unexplored.

The main target of photodamage is photosystem II (PSII), which is multisubunit membrane protein complex that catalyses the light-induced splitting of water. Photodamage of PSII is mainly due to damage to the D1 protein, which forms a heterodimer with

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the D2 protein in the reaction centre of PSII, and the subsequent rapid degradation of the D1 protein (Prasil et al., 1992). The D1 protein of thylakoid membranes has been shown to be a sensitive protein to various environmental stress conditions (Giardi et al., 1997). Photosynthetic organisms often suffer from an imbalance of synthesis/degradation of the D1 protein when exposed to stress.

The D1 protein is encoded by the *psbA* gene, which plays a critical role in replacement of injured D1 protein. Repair of photodamaged PSII requires several steps including the degradation of the damaged D1 protein, the *de novo* synthesis of D1 protein, and the incorporation of the newly synthesised D1 into PSII. Previous studies have reported that salt stress (Allakhverdiev et al., 2002), metal stress (Qian et al., 2009) and oxidative stress (Nishiyama et al., 2004) prevents the repair of photodamaged PSII by inhibiting the transcription and translation of the *psbA* genes. Wang et al. (2011) showed that drought stress remarkably inhibited the transcription of the *psbA* gene in PSII in wheat.

Aluminium (Al) toxicity can cause excessive reactive oxygen species (ROS) production, including the superoxide radical ($O_2^{\cdot-}$), the hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2). ROS that results from photosynthesis and oxidative metabolism, such as superoxide anions and hydrogen peroxide, can be involved in a number of stress responses (Foyer et al., 1994). It has been suggested that Al induces oxidative stress because this ion is involved in various processes, including enzyme activities related to ROS (Cakmak and Horst, 1991), and is associated with peroxidative damage of membrane lipids due to the stress-related increase in the production of highly toxic oxygen free radicals. Many studies have also shown that Al inhibits CO_2 assimilation and photosynthesis in many plant species. However, the effect of exogenous Spd on *psbA* transcript levels under aluminium stress has not been studied in lichens.

The aims of this study are as follows: (i) to determine the physiological response during Al stress in *Xanthoria parietina* by analysis of the chlorophyll content, the rate of chlorophyll degradation, lipid peroxidation and GR activity, (ii) to determine the effects of exogenous Spd on the transcription levels of the *psbA* gene, chlorophyll degradation and GR activity under Al stress, and (iii) to determine the effects of different concentrations of Al on the transcription levels of the *psbA* gene by semi-quantitative RT-PCR .

Results

The results of this study showed that the chlorophyll a content was significantly ($p < 0.001$) decreased by 0.5 and 1.0 mM concentration of aluminium 24 and 48 h after treatment (15.42 and 14.46, 14.55 and 11.49, respectively, as seen in Table A.1). However, the chlorophyll a content was no change significantly by exogenously adding polyamines for 24 and 48 h (20.73 and 19.23, 19.9 and 18.32, respectively, as seen in Table A.1).

The content of chlorophyll b increased significantly with increased aluminium concentration (Table A.1). The chlorophyll a/b ratio was significantly decreased after 24 and 48 h exposure to 0.5 and 1.0 mM aluminium. A positive effect of spermidine (no decrease in the chlorophyll a/b ratio) was observed in *X. parietina* after 24 and 48 h treatment with the high concentration of aluminium.

Fig. A.1 and A.2 show the malondialdehyde (MDA) content of thalli after exposure to aluminium for 24 and 48 h. Aluminium exposure significantly increased the MDA content of thalli at 0.5 and 1.0 mM for 48 h (19.5 and 30.1 nmol/g fresh wt., respectively), but not at 0.25 mM ($p < 0.01$). The increase in MDA level, as an index of lipid peroxidation, was in a concentration- and time-dependent manner. MDA production levels between Al-treated samples and exogenously Spd treated samples were also shown to be significantly different ($p < 0.01$). The 0.5- and 1.0 mM-Al

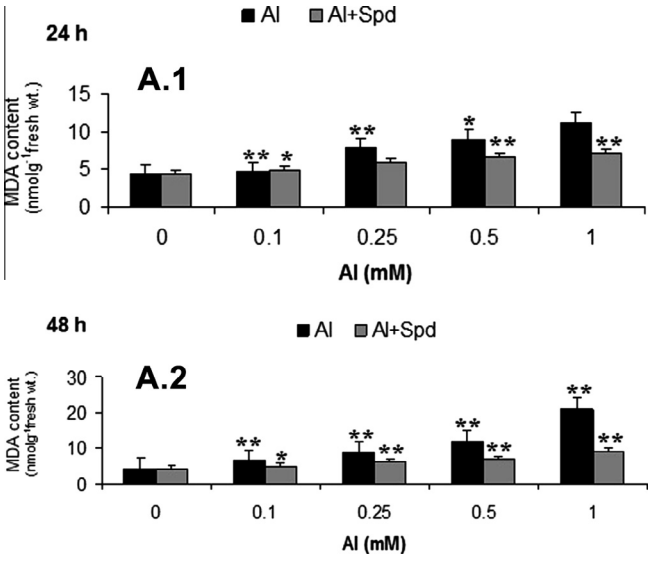

Fig. A.1 and A.2. The effects of exogenous spermidine on the MDA content of *X. parietina* thallus under different concentrations of aluminium. *Represents a statistically significant difference of $p < 0.05$ when compared with the control, **represents a statistically significant difference of $p < 0.01$.

Table A.1
Chlorophyll a (Chla), chlorophyll b (Chlb), and chlorophyll a/b (Chla/b) of the lichen *Xanthoria parietina* with control and aluminium supplement of different concentration and exogenous polyamine application groups.

	n	Chla (mg/g dw)		Chlb (mg/g dw)		Chla/b	
		24 h *X* ± SD	48 h *X* ± SD	24 h *X* ± SD	48 h *X* ± SD	24 h *X* ± SD	48 h *X* ± SD
Control	3	22.78 ± 0.03	22.19 ± 0.12	6.68 ± 0.28	6.17 ± 0.24	3.41 ± 0.08	3.59 ± 0.12
0.1 mM $AlCl_3$	3	22.85 ± 0.19	21.91 ± 0.14	6.96 ± 0.05	6.83 ± 0.04	3.28 ± 0.2	3.21 ± 0.04
0.25 mM $AlCl_3$	3	19.41 ± 0.12	18.62 ± 0.09	6.58 ± 0.08	6.98 ± 0.013	2.95 ± 0.06	2.66 ± 0.08
0.5 mM $AlCl_3$	3	15.42 ± 0.09	14.55 ± 0.18	7.77 ± 0.23	8.18 ± 0.07	1.98 ± 0.05	1.78 ± 0.08
1 mM $AlCl_3$	3	14.46 ± 0.14	11.49 ± 0.03	9.25 ± 0.26	8.74 ± 0.22	1.56 ± 0.11	1.33 ± 0.07
0.1 mM $AlCl_3$ + 1 mM Spd	3	25.30 ± 0.08	24.21 ± 0.16	6.47 ± 0.013	6.74 ± 0.11	3.91 ± 0.12	3.6 ± 0.11
0.25 mM $AlCl_3$ + 1 mM Spd	3	22.37 ± 0.06	21.68 ± 0.03	6.82 ± 0.07	7.19 ± 0.08	3.28 ± 0.08	3.013 ± 0.083
0.5 mM $AlCl_3$ + 1 mM Spd	3	20.73 ± 0.12	19.9 ± 0.012	7.52 ± 0.26	7.94 ± 0.08	2.76 ± 0.015	2.48 ± 0.03
1 mM $AlCl_3$ + 1 mM Spd	3	19.23 ± 0.05	18.32 ± 0.09	8.14 ± 0.18	8.53 ± 0.23	2.36 ± 0.075	2.17 ± 0.07
ANOVA							
F statistic		0.88	1.18	4.12	92746.11	90769.25	20396496.74
p		0.5537	0.3643	0.0060	<0.0001	<0.0001	<0.0001