Environmental and Experimental Botany 126 (2016) 44–54

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Detoxification of volcanic sulfur surplus *in planta*: Three different strategies of survival

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ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form 1 February 2016
Accepted 2 February 2016
Available online 3 March 2016

Keywords:
Volcanic H_2S/SO_2 emission
Sulfite detoxification
Sulfur metabolism
Aeolian islands

ABSTRACT

Plants face many different stress factors in their natural environment and life span. Among numerous abiotic stresses are sulfur containing volcanic gases which are highly hazardous and can enter plants *via* the stomata. As final consequence, toxic sulfite is formed inside the leaves which has to be detoxified. Controlled laboratory experiments have been performed in the past to identify the detoxification mechanisms of sulfur containing gases using model organisms. However, actual studies which investigate detoxification mechanism of H_2S/SO_2 in natural environments and include non-model organisms are missing. For this purpose plant material of eight species was sampled on the Aeolian Islands Vulcano and Lipari, at locations with harmful volcanic gases and at control sites. The collected material was analyzed to study the detoxification pathway of sulfur surplus due to exposure to H_2S/SO_2. Different reaction strategies of plants can be hypothesized: tight control of gas uptake by regulating stomata, increased synthesis of metabolites by sulfur assimilation *via* reduction of sulfite and back-oxidation of sulfite to sulfate *via* the plant molybdoenzyme sulfite oxidase followed by storage in the vacuole. The sampled plants reacted differently towards the exposure of H_2S/SO_2 with respect to closure of the stomata and overall accumulation of thiols, sulfate and/or total sulfur. Correlation analysis deciphered three different strategies of detoxification within the investigated plants: (i) Channelling of the sulfur surplus by formation of S-metabolites like thiols (reductive detoxification) and the use of mainly (ii) oxidative detoxification into sulfate with increased sulfite oxidase activity or (iii) oxidative detoxification without increased sulfite oxidase activity. One plant species did not react to sulfur surplus at all. Grouping of all tested species is consistent with their phylogenetic classification which must be strengthened in future studies.

1. Introduction

Biology of the macro-element sulfur (S) is attracting a continuously growing attention concerning cell physiology. Unbalanced levels of S compounds are considered as vulnerability factors and/or indicators of an impaired cell oxidation state in a variety of human diseases (Palego et al., 2015). Therefore, animals have to tightly control (i) resorption of S-containing compounds, mainly cysteine and methionine and (ii) elimination of toxic metabolites, especially sulfite. Plants, however, have to deal with an even more complex S-network including (i) sulfate uptake and (ii) assimilation to reduced S compounds, (iii) homeostasis of sulfate within the cell, and (iv) detoxification of harmful S compounds, which is of pivotal importance for all living organisms.

Hydrogen sulfide (H_2S) and sulfur dioxide (SO_2) are evolutionary relevant air pollutants since the development of rooted plants. They originate from large forest or peat fires and volcanic activity in nature as well as from industrial sources (Rennenberg, 1984). The toxicity of H_2S/SO_2 for organisms is based on their reaction with water thereby forming sulfite. This compound is hazardous

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http://dx.doi.org/10.1016/j.envexpbot.2016.02.007

for both animals and plants based on its nucleophilic character leading to the breakage of disulfide bridges and the degradation of proteins (Menzel et al., 1986; Heber and Hüve, 1998). In animals, the toxic sulfite originates mostly from catabolism of S-containing amino acids. Animals are mobile and can therefore escape from natural H_2S/SO_2 sources. In contrast, plants as sessile organisms had to develop strategies during evolution to cope with toxic gases on a given location or else expire.

Atmospheric H_2S/SO_2 enter plants through the stomata and are highly soluble in the apoplast. There H_2S/SO_2 are finally converted to bisulfite and sulfite within the environment of the cells (De Kok et al., 2002). Plants have to adapt to higher concentrations of H_2S/SO_2 when being constantly exposed to these pollutants in ambient air. Detoxification of these pollutants is thought to be promoted (see Fig. 1) by several mechanisms like tight control of gas uptake by regulating stomatal aperture. Additionally the increased synthesis of metabolites *via* S assimilation like the production of cysteine is controlled by modulating the activity of several enzymes especially the adenosine 5′-phosphosulfate reductase (APR) (Koprivova et al., 2008). Furthermore the back-oxidation of sulfite to sulfate is an additional option leading to the storage of sulfate in the vacuole (Buchner et al., 2004). Moreover sulfate transporters in different organs can be modulated in order to regulate the flow of S, depending on availability as well as the plant needs (Birke et al., 2015).

In this context, the oxidation of sulfite *via* the molybdoenzyme plant sulfite oxidase (pSO), localized in peroxisomes (Nowak et al., 2004), represents a safety-valve. Plant SO oxidizes sulfite with molecular oxygen as electron acceptor resulting in the formation of sulfate and hydrogen peroxide (H_2O_2). The formed hydrogen peroxide is additionally capable of detoxifying sulfite in a non-enzymatic mechanism increasing the efficiency of the pSO detoxification safety valve for sulfite removal (Lang et al., 2007). The threshold for H_2S/SO_2 toxicity varies between plant species (Kondo and Sugahara, 1978) and, furthermore, between habitats as the lack of sulfate in the soil can be compensated by plants through H_2S uptake (De Kok et al., 2007).

Fumigation experiments under controlled conditions in the laboratory using a constant exposure to SO_2 deciphered this complex regulatory network. SO_2 evokes a large scale reprogramming of the grape berry transcriptome measured by micro-array analysis (Giraud et al., 2012). RNA-deep-sequencing of *Arabidopsis thaliana* fumigated with $0.6\,\mu L\,L^{-1}$ SO_2 revealed the regulation of different enzymes involved in the metabolism/detoxification of formed sulfite (Hamisch et al., 2012). Durenkamp et al. (2007) showed that beginning with an amount of $0.6\,\mu L\,L^{-1}$ atmospheric H_2S *Allium cepa* and *Brassica oleracea* displayed reduced growth. However, a non-toxic concentration of $0.3\,\mu L\,L^{-1}$ H_2S led to increased growth in *B. oleracea*. Given these findings based on fumigation experiments one has to ask how plants react within their natural environment. Moreover, *A. thaliana* functions as a model plant and one can only hypothesize that different plant species will surely reveal diverse strategies to cope with excess S from atmospheric sources.

The aim of the present study was to identify strategies of plants coping with high concentrations of the air pollutants H_2S and SO_2 under natural conditions. For this purpose, the Aeolian Island Vulcano was chosen as a sampling site with published amounts of up to $0.5\,\mu L\,L^{-1}$ SO_2 in the atmosphere during the vegetative period (Graziani et al., 1997) originating from volcanic activity. During the sampling period of the present study the volcanic gas concentrations varied between 0.2 and $0.8\,\mu L\,L^{-1}$ (max $2\,\mu L\,L^{-1}$) H_2S and 6–8 $nL\,L^{-1}$ (max 22 $nL\,L^{-1}$) SO_2. The neighboring Island Lipari was selected to collect control species as this island shows no recent volcanic activity and lacks elevated concentrations of H_2S/SO_2 in ambient air.

2. Materials and methods

2.1. Sampling sites and H_2S/SO_2-determination

The plant material used in the present study was collected at Vulcano, a small island of volcanic origin in the Tyrrhenian Sea about 25 km north of Sicily (Italy). The island is a location of intense fumarolic and hydrothermal activity (Graziani et al., 1997). Vulcano belongs to the eight Aeolian Islands and has a size of approx. 21 km^2 (6 km wide, 8 km long) with the highest elevation of 501 m above sea level. The island Lipari located close to Vulcano was used as control sampling site.

Weather conditions are similar for Vulcano and Lipari due to their close proximity (approx. 1 km). On both islands, a typical Mediterranean flora dominates due to the etesian climate with a mild and wet winter and a warm and dry summer. The climate on the Aeolian islands is in general comparable with Messina and Palermo (on Sicily, Italy) for which long-term records (climate summary, daily average and hourly data for temperature and precipitation) are available on www.weatherbase.com/weather/city.php3?c=IT&name=Italy. Throughout the sampling period the temperatures on both islands ranged from minimum of 15 °C to maximum of 23 °C at midday. There was no rainfall during the sampling period and humidity ranged from 50 to 80% depending on the sampling site and daytime.

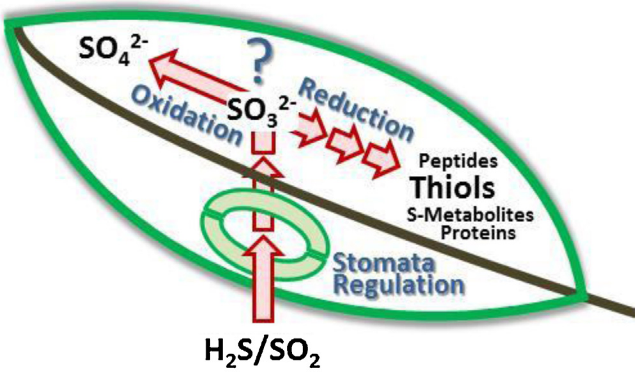

Fig. 1. Schematic representation of possible detoxification mechanisms of volcanic gases in plants.
Volcanic gases are taken up through stomata in the lower epidermis. Here the first possible reaction towards H_2S/SO_2 takes place *via* control of stomatal opening. Inside the apoplastic space H_2S/SO_2 are converted to sulfite which is either detoxified *via* oxidation forming sulfate or *via* reduction by the synthesis of S containing metabolites like thiols.