Title: Positive effects of salicylic acid pretreatment on the composition of flax plastidial membrane lipids under cadmium stress

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Abstract

Interest in use of flax (*Linum usitatissimum* L.) as cadmium (Cd)-accumulating plant for phytoextraction of contaminated soils opened up new and promising avenue toward improving tolerance of its varieties and cultivars to Cd stress. The aim of this study is to get insights into the mechanisms of Cd detoxification in cell membranes, by exploring the effects of salicylic acid (SA)-induced priming on fatty acids and lipid composition of flax plantlets, grown for 10 days with 50 and 100 μ M Cd. At leaf level, levels of monogalactosyldiacylglycerol (MGDG), phosphatidylcholine (PC), phosphatidylglycerol (PG) and neutral lipids (NL) have shifted significantly in flax plantlets exposed to toxic CdCl₂ concentrations, as compared to the control. At 100 μ M Cd, the linoleic acid (C18:2) decreases mainly in DGDG and all phospholipid species, while linolenic acid (C18:3) declines mostly in MGDG and NL. Conversely, at the highest concentration of the metal, SA significantly enhances the levels of MGDG, PG and phosphatidic acid (PA) and polyunsaturated fatty acids mainly C18:2 and C18:3. Furthermore, SA pretreatment seems to reduce the Cd-induced alterations in both plastidial and extraplastidial lipid classes but, preferentially preserves the plastidial lipids by acquiring higher levels of polyunsaturated fatty acids. These results suggest that flax plantlets pretreated with SA exhibits more stability of their membranes under Cd-stress conditions.

Key words: *Linum usitatissimum* L., salicylic acid, cadmium, membrane lipids, fatty acids, leaves.

1. Introduction

Cadmium (Cd) contamination of soils is one of the most environmental hazards that we are facing today. Industrial and municipal activities, as well as the agricultural practices can lead to a swelling of toxic levels of Cd, which will be rapidly taken up by plants (Trinchella et al. 2006). In plants, these metals affect a variety of physiological processes including photosynthesis (Seth et al. 2007; Gallego et al. 2012), metabolism-related modifications of enzyme activities (Rodriguez-Serrano et al. 2009; Andresen and Küpper 2013), gene expression (Küpper and Kochian 2010; Andresen and Küpper 2013) and the maintenance of membrane integrity for the viability of plant cells (Belkadi et al. 2010; 2013; Li et al. 2014; Liu et al. 2014). In spite of the substantial literature on the toxicology of Cd, the fundamental mechanism of its phytotoxicity has not yet been well characterized and is still lacking. Actually, cell membranes may be considered as a first target and an indicator of Cd toxicity (Hernandez and Cooke 1997; Sanz et al. 2009). Indeed, this metal is known to cause oxidative damage to membranes resulting in lipid peroxidation and loss of integrity (Ivanova et al. 2008. Li et al. 2014). Thereby, alterations in membrane lipid composition may inhibit the physiological adaptation of plants to Cd-stress conditions (Ben youssef et al. 2005; Djebali et al. 2005; Kang et al. 2014; Li et al. 2014).

Although use of phytoremediation as a biological strategy to remove pollutants from soils or make them less harmful may partially help to save the environment, some current engineering-based technologies are further required (Valderrama et al. 2012). Furthermore, interest in the microbial biodegradation of pollutants has been strengthening in recent years, by using bioremediation and biotransformation methods (Cullen and Bentley 2005; Saluja and Sharma et al. 2013). However, when we focus on human health safety, the primary way to intoxication is that Cd gets in edible parts of plants through a rapid uptake by roots (Vitoria et al. 2001; Jiang et al. 2001; Guo et al. 2007; Li et al. 2011). Thereby, providing new practical ways to prevent toxic metals from entering the food chain is also important for sustainable agriculture and health safety (Satarug et al. 2010). Besides, the improvement of plant species tolerance to metal stress needs to modulate directly or indirectly the expression of genes involved in the regulation of the amount of metals taken up from the soil by roots (Karenlampi et al. 2000). Indeed, it has been shown that there are a number of sites in the plant that could be controlled by different genes contributing to the bioaccumulation of toxic metal (Urano et al. 2010; Todaka et al. 2012). These genes govern processes that can increase the solubility of metals in the soil surrounding the roots as well as the transport proteins that move metals into root cells (Karenlampi et al. 2000; Clemens et al. 2006). From there, Cd like the other metals, enters the plant's vascular system for further transport to other parts of the plant and are ultimately deposited in leaf cells (Clemens et al. 2006). Among these practices, it has been reported that seed priming with salicylic acid (SA) before exposure to

Cd enhances plant resistance to the future Cd subjection (Ivanova et al. 2008; Krantev et al. 2008; Moussa and El-Gamal 2010; Kang et al. 2014; Li et al. 2014). In fact, SA was recognized as an exogenous plant growth regulator used to improve the natural defense mechanisms against Cd toxicity in *Oryza sativa* (Panda and Patra 2007), wheat (Agami and Mohamed 2013) and soybean (Li et al. 2014). In order to understand the mechanisms by which this improvement may have been reached; we really need to know to what extent an exogenous SA pretreatment can induce changes in fatty acid composition of the major lipid components of cell membranes. Only a few recent studies have examined the effects of SA pretreatment on membrane lipid metabolism under Cd stress. Most of these reports have been focused mainly on lipid peroxidation (Choudhury and Panda 2004; Guo et al. 2007; Zhang and Chen 2011) and total fatty acid composition of stressed plants (Ivanova et al. 2008). As it is evident that Cd exposure led to serious changes in the lipid membranes, the present study aims to test the hypothesis that using seed priming with SA will induce positive modifications in membrane lipid composition of *Linum usitatissimum* and the induced protection is associated with SA role as stabilizer of membrane integrity to improve flax resistance to Cd stress.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of flax (*Linum usitatissimum* cv. Viking), were soaked for 8 h in SA solutions as previously shown in Belkhadi et al. (2010). Then they were germinated for four days at 25 °C in the dark and grown hydroponically in a continuously aerated nutrient solution (pH 5.5) containing 1 mM MgSO₄, 2.5 mM Ca (NO₃)₂, 1 mM KH₂PO₄, 2 mM KNO₃, 2 mM NH₄Cl, 50 mM EDTA–Fe–K, 30 mM H₃BO₃, 10 mM MnSO₄, 1 mM ZnSO₄, 1 mM CuSO₄ and 30 mM (NH₄)₆Mo₇O₂₄. The hydroponic medium was buffered with HCl/KOH and changed twice per week. After growing for 2 days, CdCl₂ was added in 3 concentrations (0, 50 and 100 µM) during 10 days. Five replicates were, in individual 6 l black beakers, made for control and Cd treatments. Plantlets were grown in a growth chamber at a day/night cycle of 16 h/8 h, at 23 °C/18 °C, respectively, a relative humidity close to 75% and a light intensity of 200 µmol photons m⁻² s⁻¹. Three independent culture experiments were performed.

2.2. Membrane stability index

Membrane stability index (MSI) is a marker for oxidative stress that was determined by recording the electrical conductivity of leaf tissues in double distilled water at 40 and 100 °C as shown in Belkhadi et al. (2010). In brief,

leaf samples (100 mg) were cut into discs of uniform size and taken in test tubes containing 10 ml of double distilled water in two sets. One set was kept at 40 °C for 30 min and another set at 100 °C in boiling water bath for 15 min and their respective electric conductivity's C1 and C2 were measured by conductivity meter. The MSI was determined using the following formula: $MSI = (1 - (EC1/EC2)) \times 100$.

2.3. Lipid extraction and analysis

Lipids in roots were extracted according to Garcés and Mancha (1993) using 30 mg of leaves harvested after Cd treatment of SA pretreated and non pretreated seedlings. The total lipids (TL) were separated into phospholipids (PL), galactolipids (GL) and neutral lipids (NL) on silica-gel thin layer chromatography (TLC) plates (Merck, Darmstadt, Germany), according to Nichols (1965). To visualize the lipid bands, the plates were sprayed with I₂ vapor. Lipid classes were identified against lipid standards using specific stains for PL and GL. Fatty acid contents were separated and quantified using an integrator (Model 3390 A, Hewlett-Packard, USA) and a fused silica capillary column BPX 70 (SGE, Austin, TX, USA), length 50 m, 0.22 mm i.d., 0.25 mm film thickness. The samples of fatty acid methyl esters (FAMES) dissolved in hexane were injected using an auto sampler CP 9050. PL, GL and LN were injected manually. Helium was used as the carrier gas at a pressure of 150 kPa and nitrogen was used as make-up gas at a flow rate of 30 ml min⁻¹. Detector and injector temperatures were at 250 and 230 °C, respectively. The peak areas were identified by comparing their retention times and responses with those of a standard FAMES mixture, GLC-68 A (Nu-Chek Prep, Elysian, USA).

2.4. Statistical analysis

Statistical calculations were performed with SPSS-17 statistical software. Mean difference comparison among different treatments was done by analysis of variance and Tukey's (HSD) test at a 0.05 probability level.

3. Results

3.1. Effect of SA pretreatment on leaf membrane stability under Cd stress

Cell membrane stability is an indicator to differentiate stress tolerant cultivars of crops and higher membrane stability could be correlated with oxidative stress tolerance. The MSI shows completely different patterns of response in the leaves exposed to the highest Cd concentration in the presence or absence of SA pretreatment (Fig. 1). Under normal conditions, SA-pretreated leaves exhibit significant improvement in the MSI that is, at 250 µM SA, 20.3% higher over the control plantlets. The exposure of the flax to Cd stress (50 and 100 µM) significantly decreases the MSI values. These decreases are by about 14% and 44.4% over control at 50 and

100 μ M, respectively. However, within both Cd concentrations, SA pretreatment leads to the maintenance of the stability of membranes and that in a concentration dependent manner (Fig. 1). Thus, at 100 μ M Cd-treated plantlets, SA (250 or 1000 μ M) enhances the MSI in leaves by about 23.6% and 51.3%, respectively, compared to control.

3.2. Effect of SA pretreatment on lipid contents of leaf membrane under Cd stress

In control plants, significant differences in the lipid composition of leaves are observed (Figs. 2-4). In galactolipids (GL), most important constituents of plant membranes, the proportion of monogalactosyldiacylglycerol (MGDG) is higher than that of digalactosyldiacylglycerol (DGDG) (33 and 25% of total lipids, respectively) (Fig. 2). However, the most abundant phospholipid (PL) in leaves is the phosphatidylcholine (PC) (Fig. 3).

The application of increasing doses of CdCl₂ (0, 50 and 100 μ M), results in a net reduction in the levels of GL in flax leaves which is generated by a significant decrease in percentages of MGDG (Fig. 2). At 50 or 100 μ M Cd, this decrease is by about 21% over control. Figure 3 shows that only 2 molecules of PL (PC and PG) undergo a gradual decrease within both Cd concentrations (50 and 100 μ M). Thus, at 100 μ M Cd, the percentages of PC and PG are diminished by 35% and 39.3%, respectively, compared to control. The levels of NL are also significantly reduced by Cd stress (Fig. 4). At 100 μ M Cd, there is a decrease by 25% in their amounts, compared to the control.

The results show that in leaf of Cd-contaminated flax, SA seems probably correct the metal negative effects on membranes. In fact, remarkable increase is noticed in the levels of chloroplast lipids (MGDG and PG) at 100 μ M Cd, (Figs. 2 and 3) whereas a significant increase (+ 33.3%) in the level of PA is observed in leaves treated with 250 μ M + 50 μ M Cd (Fig. 3). These increases are SA- and Cd- dose dependent. It is important to report that at the highest metal concentration, the most prominent effect is at 1000 μ M SA; 14% of increase in the percentage of MGDG is noticed whereas SA (250 or 1000 μ M) enhances by about 16% the PG percentage, in comparison with non SA-pretreated leaves (Figs. 2 and 3). However, upon the SA pretreatment, there is no modification in the NL percentages in flax leaves under normal or stress conditions (Fig. 4).

3.3. Effect of SA pretreatment on fatty acid composition of leaf membrane under Cd stress

The changes in fatty acid composition due to Cd-stress differ between individual lipids (Tables 1-5). Indeed, we note that for the constituent fatty acids of MGDG molecule, particularly at the dose (100 μ M Cd), there is a decrease in C18:2 and C18:3 at the extent of that of C16:0 and C18:0 (Table 1). Regarding composition of the different PL components, the results show that the presence of 100 μ M Cd in the medium causes a fall in rates of C18:3 and C18:2 in favor of C16:0, at all the molecules of PL (Tables 2-4). In PG molecule, the rate of C16:1 *trans*

(characteristic fatty acid of the molecule) also falls following the application of Cd (Table 2). Composition of NL is significantly affected by Cd. Table 5 shows that this change may mainly due to the sharp reduction in the percentage of C18:3 offset by an increase in the C16:0 rate.

In plantlets contaminated by Cd, SA pretreatment also modifies the composition of leaf fatty acids. Thus, for the molecule of MGDG, we see that in addition to the increasing amounts of C18:2, the action of SA is essentially reflected by a significant stimulation in the percentages of C18:3 (Table 1). In fact, in flax seedlings pretreated with 250 μ M and 1000 μ M SA, the rate of C18:3 increases by 47% and 63%, compared to non-pretreated seedlings, respectively, at 100 μ M Cd (Table 1). As for MGDG, the SA-effect on PL molecules (PG and PA) is also characterized by a common increase in the proportions of polyunsaturated fatty acids, C18:2 and C18:3 (Tables 2 and 4). As a result, it becomes evident that in flax leaves, SA rather acts as an inhibitor of Cd toxicity mostly in chloroplastic lipids where it induces significant stimulations in both percentage and fatty acid composition of individual lipids. In contrast to GL and PL, the effects of SA pretreatment on NL levels and fatty acid composition under Cd stress are apparently smaller.

4. Discussion

Biological membranes are the structures that mostly formed by lipids (Cacas et al. 2012). These are shifting with the external environment, and may be considered as the first target of toxic metals (Mishra and Choudhuri 1999). Plant membranes may also be responsive to other abiotic and biotic stresses, including salt (Cuin and Shabala 2007), drought (Huang et al. 2008; Kamthan et al. 2012), heat treatment (Whitaker 1991), fungal (Avis et al. 2001) and viral infections (Lorizate and Kräusslich 2011). Undoubtedly, various toxic effects of Cd on the leaf membrane lipids have been previously reported (Mishra and Choudhuri 1999; Ben youssef et al. 2005; Djebali et al. 2005; Le Guédard et al. 2012). However, in this study, we have found that in the leaves of flax plantlets pretreated with SA, the amounts of chloroplastic lipids that has been evaluated from the amount of total fatty acids, was positively changed under Cd stress, and was considerably dependent on both SA and Cd doses.

Our results indicated a dramatic decrease in the levels of MGDG, PC, PG and NL in flax leaves treated for 10 days with 50 and 100 μ M Cd. In fact, Cd stress is known to enhance lipoxxygenase activity, which is responsible for inducing hydro-peroxidation of unsaturated fatty acids (Djebali et al. 2005; Kuznetsova et al. 2008). Furthermore, Cd is also implicated in the indirect production of reactive oxygen species (ROS) inside cells that leads to the peroxidation of membrane lipids (Guo et al. 2007). However, at 100 μ M Cd, we showed that 8-h SA pretreatment increased the levels of chloroplastic lipids (MGDG and PG) and those of PA in leaves. Actually,

MGDG is considered as an indispensable lipid for photosynthesis, because it regulates the supramolecular structure of photosystem II (PSII) complex (Cacas et al. 2012). This increase in the MGDG amount can be interpreted by a protection of PSII complex stability (Aronsson et al. 2008; Mizusawa and Wada 2012). Furthermore, the SA-induced changes in the proportions of PG and PA in Cd-treated flax leaves may help to maintain the membrane fluidity and preserve the intrinsic-membrane protein activities (Kim et al. 2006; Fagioni et al. 2009) and can also provoke an expansion of the total membrane area of the Cd-treated cells in particular the vacuolar membrane. In fact, it is admitted that the vacuole is the site for the accumulation of Cd (Clemens et al. 2006); thus, the adjustment of compartmentalization can be considered as a very important mechanism towards flax enhancement of tolerance to toxic metal and detoxification with non protein thiols (Belkadhi et al. 2012). Then again, SA could be involved in the expression of specific proteins or defense-related enzymes (Zawoznik et al. 2007). On the other hand, it has been shown that SA acts as a signaling molecule and regulates the biogenesis of chloroplasts (Uzunova and Popova 2000) and photosynthetic activity (Fariduddin et al. 2003). Besides, endogenous SA movement across membranes is regulated by ROS and Ca^{2+} (Chen et al. 2001). Our results indicated that before Cd exposure, SA pretreatment increased the MSI of flax leaves (Fig. 1). This could be due to the fact that SA may act as inner balance of Ca^{2+} (homeostasis) and antioxidant system (Wang and Li 2006). Moreover, supplementation of tobacco cell suspension culture with SA resulted in a *de novo* induction of SA excretion across the membrane which was mediated by the generation of ROS and activation of a cascade of Ca^{2+} signaling and protein phosphorylation (Chen et al. 2001).

Analysis of the lipid composition of flax leaf membranes also showed that exposure to Cd affected the relative abundance of various fatty acids, especially, the polyunsaturated ones (C18:2 and C18:3) (Tables 1-5). Cd is not a transition metal and hence will not be able to directly generate damaging oxygen species (Li et al. 2014). Thus, in examination of several studies on Cd-mediated lipid peroxidation, there are many causes to deduce that the decrease in unsaturated fatty acid levels, mostly those of C18:2 and C18:3, observed in the lipid molecular species of treated plantlets, might be related to indirect reaction of ROS with unsaturated lipids. Likewise Cd is the most powerful catalyst of free radical formation and Cd-ions themselves can trigger indirectly oxidative damage of membrane polyunsaturated lipids (Le Guédard et al. 2012). However, the decrease of polyunsaturated fatty acid amounts does not eliminate an inhibition of fatty acid desaturases by Cd (Mazliak et al. 1980). These results corroborate previous studies performed on lettuce and tomato seedlings, which showed that the percentage of tri-unsaturated fatty acids in leaves decreased while the percentages of C18:2, C18:1 and C18:0 increased after 14 days of growth in contaminated soil (Verdoni et al. 2001; Le Guédard et al. 2012). Similar results have also been observed in tomato leaves grown hydroponically under Cd stress (Djebali et al. 2005).

The MSI has been used as indicator of oxidative injuries and Cd tolerance in plants (Andresen and Küpper 2013). In fact, it has been suggested that increase in membrane stability reflects the limits of lipid peroxidation caused by ROS (Li et al. 2014). Under Cd stress, MSI was higher in SA-pretreated leaves than non pretreated ones (Fig. 1). In our previous work we have shown that Cd treatment of flax plantlets increased formation of hydrogen peroxide (H₂O₂) and traced their negative effects on lipid peroxidation and membrane permeability (Belkadi et al. 2010; 2013). Increased (H₂O₂) accumulation and lipoperoxidation due to Cd stress resulted in significant decrease in MSI values, however, the lesser degree of membrane damage (as indicated by MSI) in SA pretreated plantlets indicated their higher capacity for the scavenging of Cd-generated ROS. Liu et al. (2014) reported that exogenous SA alleviated the toxicity generated by salt stress in cotton seedlings by a reduction in ROS level and lipid peroxidation. These authors also concluded that SA facilitated the membrane transport and function by decreasing leaf electrolyte leakage, improving ion absorption, activating the osmotic-regulated substances metabolic and also by alleviating the NaCl-induced inhibition of H⁺-ATPase in plasma membrane. Additionally, under Cd-stress conditions, Li et al. (2014) showed that SA might act as membrane stabilizer as well as ROS scavenger.

Concerning fatty acid composition of SA pretreated leaves, the results showed that the fatty acid composition of MGDG was in discrete **increases** of C18:3 and **C18:2** percentages at 100 µM Cd. This response can be explained by a probable role of SA on inducing stearyl-ACP-desaturase, which is known to play a key position in C18 fatty acid biosynthesis (Kachroo et al. 2007). In fact, Kachroo et al. (2003) have analyzed the *Arabidopsis ssi2* mutant, which encodes a defective stearyl-ACP-desaturase and consequently accumulates high levels of 18:0 and low levels of 18:1 fatty acids. Furthermore, they also showed that altered 18:1 fatty acid content in the *ssi2* mutants has an impact on SA- and **jasmonate**-mediated defense signaling.

Similarly, the subject of whether or not different PL classes and NL behave differently to GL components, regarding their fatty acid composition in SA pretreated leaves under Cd stress. Among the different fatty acids, it has been notable that SA increased mostly the PG-18:2, 18:3 and PA-18:2, 18:3. In plants, it is well assumed that phospholipases D (PLD) have been implicated in abiotic stress responses (Mane et al. 2007) and are major components of signaling pathways in plant responses to some stresses and hormones (Rainteau et al. 2012). Besides, Krinke et al. (2009) established that PA biosynthesis is also activated in response to SA. Despite the physiological importance of these responses, it has been shown that PG, major GL in chloroplast membranes, may be a PLD substrate. Thus, the SA-induced accumulation of PG and PA polyunsaturated acids, under Cd stress, could be an indication that there is possible influence of SA on PLD products inside chloroplasts. In *Arabidopsis* cells, Rainteau

et al. (2012) by studying the signaling role of PLD and analyzing the composition of its PA products in the presence and absence of SA have shown that PLD action in response to SA is not due to the production of a stress-specific molecular species, but that the level of PLD products is important. Interestingly, the over-representation of 16:0/18:2- and 16:0/18:3-species in PLD products when compared to putative substrates might be related to a regulatory role of the heterogeneous distribution of GL in membrane sub-domains (Rainteau et al. 2012).

Conclusion

In conclusion, notable differences occurred between non pretreated and pretreated flax plantlets with SA in their membrane lipid changes under Cd-stress conditions. We have investigated the effects of exogenous SA on the leaf lipid membrane composition after subjection to high Cd treatments. Marked changes in membrane composition were observed after 10 days of exposure to Cd. Some specific SA-induced changes have been noticed and were clearly dose-dependent. It is also debatable whether these changes represent a protection against the toxic effects of Cd, or from SA-initiated action to enhance membrane stabilization. We propose that the lower loss of membrane stability in SA-pretreated flax leaves under Cd stress may be related to a minor rate of the lipid catabolism and/or oxidation. Use of SA pretreatment may be an effective strategy to protect mostly the chloroplastic lipids and would be promising in controlling growth and improving photosynthetic capacity to allow plants to adapt to their environmental conditions.

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Tables

Table 1

Effects of SA on fatty acid composition (% of TL) in leaf monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) of 16-d-old flax plantlets treated with different Cd treatments.

SA	Cd	Fatty acids					
(μM)	(μM)	<i>C16:0</i>	<i>C16:1 cis</i>	<i>C18:0</i>	<i>C18:1</i>	<i>C18:2</i>	<i>C18:3</i>
MGDG							
0	0	30.68±0.21 c	Tr	2.19±0.01 a	4.26±0.02 c	6.56±0.07 b	53.54±0.50 b
0	50	37.95±0.02 d	Tr	5.81±0.29 b	3.83±0.14 b	5.52±0.02 a	49.13±0.12 a
0	100	36.78±0.44 d	Tr	9.24±0.16 d	0.85±0.10 a	4.65±0.02 a	43.48±0.21 a
250	0	32.99±0.02 c	Tr	2.59±0.21 a	3.34±0.21 c	5.39±0.40 a	56.47±0.77 c
250	50	23.63±0.11 b	Tr	6.21±0.09 bc	2.62±0.12 b	7.30±0.11 c	60.32±0.11 c
250	100	22.10±0.29 b	Tr	7.33±0.01 c	0.45±0.10 a	6.67±0.01 b	64.01±0.13 d
1000	0	30.12±0.01 c	Tr	4.07±0.09 b	2.97±0.30 b	5.85±0.19 ab	57.57±0.01 c
1000	50	21.45±0.19 b	Tr	7.72±0.04 c	2.23±0.01 b	9.57±0.58 d	59.17±0.20 c
1000	100	14.56±0.01 a	Tr	7.33±0.01 c	0.46±0.20 a	7.39±0.11 c	70.81±0.01 d
DGDG							
0	0	6.07±0.04 a	Tr	1.27±0.03 a	4.23±0.11 a	32.59±0.24 e	50.87±0.18 b
0	50	13.65±0.01 a	Tr	2.59±0.02 a	9.89±0.01 c	17.11±0.05 c	56.78±0.01 c
0	100	12.25±0.31 b	Tr	4.49±0.10 b	11.38±0.12 c	22.81±0.11 c	49.08±0.21 b
250	0	9.69±0.02 a	Tr	4.10±0.14 b	2.49±0.01 a	36.01±0.02 f	54.76±0.06 bc
250	50	15.08±0.05 b	Tr	1.52±0.04 a	8.80±0.01 b	18.59±0.01 c	56.03±0.01 c
250	100	14.62±0.17 b	Tr	5.45±0.10 c	12.05±0.02 d	24.10±0.29 d	43.79±0.03 a
1000	0	13.65±0.01 b	Tr	1.07±0.09 a	6.03±0.01 b	17.11±0.05 b	47.21±0.10 b
1000	50	13.21±0.01 a	Tr	3.67±0.01 b	10.08±0.03 c	20.69±0.02 d	52.36±0.01 bc
1000	100	18.77±0.01 c	Tr	5.67±0.01 c	12.03±0.01 d	22.56±0.01 c	41.00±0.24 a

^aData are means of three independent experiments ($\pm$ SE).

^bMeans with different letters indicate statistically different results at $P \leq 0.05$, according to Tukey's (HSD) test, (Tr, trace.

Table 2

Effects of SA on fatty acid composition (% of TL) in leaf phosphatidylglycerol (PG) of 16-d-old flax plantlets treated with different Cd treatments.

SA (μ M)	Cd (μ M)	Fatty acids						
		<i>C16:0</i>	<i>C16:1 cis</i>	<i>C16:1 trans</i>	<i>C18:0</i>	<i>C18:1</i>	<i>C18:2</i>	<i>C18:3</i>
0	0	33.35 $\pm$ 0.02 a	Tr	20.67 $\pm$ 0.07 c	7.44 $\pm$ 0.01 c	7.58 $\pm$ 0.21 c	5.17 $\pm$ 0.07 b	23.39 $\pm$ 0.17 b
0	50	35.90 $\pm$ 0.10 b	Tr	21.45 $\pm$ 0.04 d	7.15 $\pm$ 0.12 c	7.05 $\pm$ 0.08 bc	7.72 $\pm$ 0.01 c	21.40 $\pm$ 0.09 a
0	100	40.07 $\pm$ 0.01 c	Tr	16.45 $\pm$ 0.10 a	5.27 $\pm$ 0.01 b	8.86 $\pm$ 0.26 d	4.11 $\pm$ 0.11 a	20.51 $\pm$ 0.13 a
250	0	32.27 $\pm$ 0.02 a	Tr	20.46 $\pm$ 0.08 bc	7.38 $\pm$ 0.02 c	7.25 $\pm$ 0.50 bc	4.51 $\pm$ 0.03 a	25.39 $\pm$ 0.11 b
250	50	30.91 $\pm$ 0.11 a	Tr	20.00 $\pm$ 0.11 d	6.53 $\pm$ 0.11 bc	6.75 $\pm$ 0.01 b	8.50 $\pm$ 0.98 d	27.53 $\pm$ 0.87 c
250	100	43.07 $\pm$ 0.01 c	Tr	17.05 $\pm$ 0.01 b	3.15 $\pm$ 0.10 a	8.28 $\pm$ 0.38 d	6.06 $\pm$ 0.98 b	29.98 $\pm$ 1.00 d
1000	0	33.00 $\pm$ 0.28 a	Tr	20.90 $\pm$ 0.19 c	7.31 $\pm$ 0.21 c	7.22 $\pm$ 0.22 bc	4.44 $\pm$ 0.11 a	24.37 $\pm$ 0.16 b
1000	50	32.04 $\pm$ 0.07 a	Tr	19.32 $\pm$ 0.03 bc	7.00 $\pm$ 0.12 c	5.65 $\pm$ 0.33 a	8.30 $\pm$ 0.08 d	27.85 $\pm$ 0.10 c
1000	100	38.68 $\pm$ 1.09 bc	Tr	17.55 $\pm$ 0.97 b	5.30 $\pm$ 0.08 b	3.31 $\pm$ 0.58 a	6.63 $\pm$ 1.12 b	29.46 $\pm$ 0.08 d

^aData are means of three independent experiments ($\pm$ SE).

^bMeans with different letters indicate statistically different results at $P \leq 0.05$, according to Tukey's (HSD) test, (Tr, trace

Table 3

Effects of SA on fatty acid composition (% of TL) in leaf phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of 16-d-old flax plantlets treated with different Cd treatments.

SA	Cd	Fatty acids					
(μM)	(μM)	<i>C16:0</i>	<i>C16:1 cis</i>	<i>C18:0</i>	<i>C18:1</i>	<i>C18:2</i>	<i>C18:3</i>
PC							
0	0	37.64±0.11 b	Tr	4.38±0.35 a	5.74±0.19 c	26.82±0.19 c	25.44±0.09 b
0	50	43.75±0.19 c	Tr	5.00±0.01 ab	5.84±0.08 c	21.89±0.21 b	23.54±0.09 ab
0	100	50.15±0.10 e	Tr	5.33±0.10 ab	2.18±0.10 a	18.33±0.01 a	24.05±0.22 ab
250	0	36.59±0.21 b	Tr	5.56±0.19 b	6.63±0.06 d	24.60±0.02 bc	26.54±0.06 b
250	50	46.63±0.39 cd	Tr	5.05±0.01 ab	4.45±0.10 b	20.56±0.12 b	23.32±0.19 a
250	100	55.64±0.94 f	Tr	5.94±0.05 b	1.78±0.01 a	15.81±0.97 a	20.84±0.03 a
1000	0	38.90±0.03 bc	Tr	5.50±0.06 b	6.31±0.02 d	23.71±0.11 b	25.59±0.22 b
1000	50	48.42±0.28 d	Tr	4.33±0.25 a	3.82±0.28 b	20.66±0.34 b	22.79±0.09 a
1000	100	33.35±0.02 a	Tr	7.44±0.01 c	2.67±0.07 ab	15.17±0.07 a	22.37±0.46 a
PE							
0	0	30.44±0.03 a	Tr	10.06±0.11 b	10.85±0.07 b	20.23±0.25 d	28.44±0.11 e
0	50	35.20±0.01 c	Tr	10.42±0.17 b	12.14±0.07 b	18.54±0.07 c	23.72±0.04 c
0	100	37.99±0.98 d	Tr	11.24±0.01 b	13.68±0.98 c	17.05±0.01 c	20.05±1.98 b
250	0	30.77±0.11 a	Tr	9.51±0.09 a	10.43±0.09 a	21.07±0.11 e	28.24±0.04 e
250	50	36.30±0.01 c	Tr	12.28±0.06 c	13.04±0.01 c	16.27±0.01 b	22.13±0.05 b
250	100	41.44±0.10 f	Tr	13.60±0.02 e	13.22±0.04 c	15.53±0.05 b	16.21±0.02 a
1000	0	33.37±0.09 b	Tr	10.23±0.07 b	11.22±0.09 b	19.83±0.05 d	25.37±0.06 d
1000	50	39.29±0.47 e	Tr	13.42±0.02 e	12.14±0.05 b	13.49±0.87 a	21.68±0.09 b
1000	100	39.90±0.80 e	Tr	14.02±0.78 f	13.29±0.18 c	15.72±0.10 b	17.07±0.10 a

^aData are means of three independent experiments ($\pm$ SE).

^bMeans with different letters indicate statistically different results at $P \leq 0.05$, according to Tukey's (HSD) test, (Tr, trace

Table 4

Effects of SA on fatty acid composition (% of TL) in leaf **phosphatidylinositol (PI)** and **phosphatidic acid (PA)** of 16-d-old flax plantlets treated with different Cd treatments.

SA	Cd	Fatty acids					
(μM)	(μM)	<i>C16:0</i>	<i>C16:1 cis</i>	<i>C18:0</i>	<i>C18:1</i>	<i>C18:2</i>	<i>C18:3</i>
PI							
0	0	24.48±0.01 a	Tr	3.50±0.98 b	18.70±0.01 a	17.95±0.07 c	35.39±1.07 d
0	50	30.48±0.09 b	Tr	6.93±0.09 c	19.71±0.11 b	15.53±0.05 b	27.37±0.02 c
0	100	25.66±0.98 a	Tr	11.36±0.01 e	23.66±0.01 d	15.69±0.05 b	23.65±1.05 b
250	0	28.23±0.09 b	Tr	2.25±0.09 a	17.76±0.19 a	18.12±0.04 d	33.66±0.03 d
250	50	32.95±0.02 c	Tr	9.71±0.04 d	19.58±0.19 b	13.44±0.43 a	24.33±0.68 b
250	100	37.48±0.11 e	Tr	12.26±0.09 f	18.40±1.02 a	18.40±0.04 d	11.22±0.06 a
1000	0	23.73±0.52 a	Tr	6.38±0.30 c	19.11±0.19 b	17.13±0.04 c	33.67±0.01 d
1000	50	35.14±0.01 d	Tr	9.62±0.03 d	20.32±0.04 c	12.86±0.21 a	22.08±0.28 b
1000	100	37.51±0.31 e	Tr	13.17±0.26 g	17.23±0.39 a	11.15±0.29 a	20.95±0.68 b
PA							
0	0	28.61±0.89 b	Tr	7.55±0.87 a	10.02±0.09 c	15.95±0.06 d	37.89±0.17 b
0	50	32.76±0.08 c	Tr	10.12±0.11 b	12.65±0.09 c	11.41±0.11 a	33.08±0.01 a
0	100	33.07±0.01 c	Tr	10.85±0.10 c	11.34±0.01 c	13.04±0.01 b	31.72±0.13 a
250	0	26.76±0.01 a	Tr	8.50±0.01 b	10.26±0.02 c	13.13±0.05 b	41.37±0.09 d
250	50	29.45±0.28 b	Tr	8.34±0.11 b	8.60±0.63 b	13.93±0.12 b	39.69±0.12 c
250	100	30.23±0.01 e	Tr	9.89±0.89 b	9.18±0.01 b	14.23±0.98 c	37.48±1.98 b
1000	0	30.77±0.01 c	Tr	7.95±0.01 a	11.17±0.01 c	15.07±0.04 c	35.06±0.01 b
1000	50	33.11±0.09 f	Tr	7.60±0.11 a	6.81±0.11 a	12.84±0.02 b	37.66±0.07 b
1000	100	26.63±0.01 a	Tr	13.45±0.10 d	10.22±0.01 c	15.57±0.01 c	35.15±0.13 b

^a Data are means of three independent experiments ($\pm$ SE).

^b Means with different letters indicate statistically different results at $P \leq 0.05$, according to Tukey's (HSD) test, (Tr, trace

Table 5

Effects of SA on fatty acid composition (% of TL) in leaf neutral lipid (NL) of 16-d-old flax plantlets treated with different Cd treatments.

SA (μ M)	Cd (μ M)	Fatty acids					
		<i>C16:0</i>	<i>C16:1 cis</i>	<i>C18:0</i>	<i>C18:1</i>	<i>C18:2</i>	<i>C18:3</i>
0	0	37.17 $\pm$ 0.01 a	Tr	7.78 $\pm$ 0.03 a	13.73 $\pm$ 0.19 e	12.46 $\pm$ 0.12 b	28.88 $\pm$ 0.09 c
0	50	41.15 $\pm$ 0.01 ab	Tr	7.55 $\pm$ 0.69 a	14.13 $\pm$ 0.20 e	14.72 $\pm$ 0.33 c	22.46 $\pm$ 0.17 b
0	100	40.83 $\pm$ 0.97 a	Tr	9.10 $\pm$ 0.02 c	13.45 $\pm$ 0.63 c	17.45 $\pm$ 0.63 e	19.18 $\pm$ 0.30 a
250	0	36.58 $\pm$ 0.02 a	Tr	7.94 $\pm$ 0.09 a	13.26 $\pm$ 0.10 c	13.56 $\pm$ 0.02 b	28.67 $\pm$ 0.19 c
250	50	41.84 $\pm$ 0.11 ab	Tr	9.50 $\pm$ 0.30 c	12.85 $\pm$ 0.21 c	16.10 $\pm$ 0.18 d	19.73 $\pm$ 0.19 a
250	100	48.71 $\pm$ 0.57 bc	Tr	11.50 $\pm$ 1.18 d	6.81 $\pm$ 1.04 a	10.82 $\pm$ 1.03 a	22.17 $\pm$ 0.32 b
1000	0	42.03 $\pm$ 0.01 ab	Tr	8.92 $\pm$ 0.01 b	13.25 $\pm$ 0.04 c	13.57 $\pm$ 0.02 b	22.24 $\pm$ 0.02 b
1000	50	42.94 $\pm$ 0.11 ab	Tr	9.52 $\pm$ 0.11 c	13.22 $\pm$ 0.09 c	15.79 $\pm$ 0.23 d	18.55 $\pm$ 0.14 a
1000	100	46.32 $\pm$ 1.10 bc	Tr	8.63 $\pm$ 0.14 b	10.28 $\pm$ 0.24 b	14.28 $\pm$ 0.24 c	20.50 $\pm$ 0.74 b

^aData are means of three independent experiments ($\pm$ SE).

^b Means with different letters indicate statistically different results at $P \leq 0.05$, according to Tukey's (HSD) test.

^c TL: total lipids; Tr: trace

Figures

Figure 1

Figure 2

Figure 3

Figure 4

Figure captions

Fig. 1 Membrane stability index of leaves of SA-pretreated flax plantlets grown in hydroponic cultures and subjected for 10 days to increased CdCl_2 concentrations. Results are the means of 5 replicate experiments $\pm$ SE. Error bars with common letters are not significantly different at $P \leq 0.05$, according to Tukey's test.

Fig. 2 Glycolipid classes of leaves of SA-pretreated flax plantlets grown in hydroponic cultures and subjected for 10 days to increased CdCl_2 concentrations. Results are the means of 5 replicate experiments $\pm$ SE. Error bars with common letters are not significantly different at $P \leq 0.05$, according to Tukey's test. MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol.

Fig. 3 Phospholipid classes of leaves of SA-pretreated flax plantlets grown in hydroponic cultures and subjected for 10 days to increased CdCl_2 concentrations. Results are the means of 5 replicate experiments $\pm$ SE. Error bars with common letters are not significantly different at $P \leq 0.05$, according to Tukey's test. PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PA, phosphatidic acid; PI, phosphatidylinositol.

Fig. 4 Neutral lipid (NL) class of leaves of SA-pretreated flax plantlets grown in hydroponic cultures and subjected for 10 days to increased CdCl_2 concentrations. Results are the means of 5 replicate experiments $\pm$ SE. Error bars with common letters are not significantly different at $P \leq 0.05$, according to Tukey's test.