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Salicylic acid and photosynthesis: signalling and effects

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Abstract

Salicylic acid (SA) is a well-known signalling molecule playing a role in local and systemic acquired resistance against pathogens as well as in acclimation to certain abiotic stressors. As a stress-related signalling compound, it may directly or indirectly affect various physiological processes, including photosynthesis. The effects of exogenously applied SA on plant

physiological processes under optimal environmental conditions are controversial. Several studies suggest that SA may have a positive effect on germination or plant growth in various plant species. However, SA may also act as a stress factor, having a negative influence on various physiological processes. Its mode of action depends greatly on several factors, such as the plant species, the environmental conditions (light, temperature, etc.) and the concentration. Exogenous SA may also alleviate the damaging effects of various stress factors, and this protection may also be manifested as higher photosynthetic capacity. Unfavourable environmental conditions have also been shown to increase the endogenous SA level in plants. Recent results strongly suggest that controlled SA levels are important in plants for optimal photosynthetic performance and for acclimation to changing environmental stimuli. The present review discusses the effects of exogenous and endogenous SA on the photosynthetic processes under optimal and stress conditions.

Running title: Salicylic acid and photosynthesis

Keywords: Fluorescence quenching, Net photosynthesis, Oxidative stress, Salicylic acid, Stomatal conductivity, Stress responses

Abbreviations: CA: cinnamic acid, C_i: Intercellular CO_2 concentration, F_m: maximum chlorophyll-a fluorescence at dark adapted state, ,F_v: variable chlorophyll-a fluorescence at dark adapted state, g_s: stomatal conductivity, ICS: isochorismate synthase, NPQ: non-photochemical fluorescence quenching, oHCA: *ortho*-hydroxycinnamic acid, PAL: phenylalanine ammonia lyase, PEPC: phosphoenolpyruvate carboxylase, P_N: net

photosynthetic rate, PS: Photosystem, ROS: reactive oxygen species, SA: salicylic acid; TL: thermoluminescence; Φ_{PSII}: actual photochemical efficiency of PSII

Introduction

Phytohormons contribute to all aspects of plant responses towards biotic and abiotic stimuli. They are also indicators of plant stress responses upon variation of light, salinity, temperature, radiation, pollutions (Vogt 2010; Kosova et al. 2012; Majláth et al. 2012). Salicylic acid (SA) is a monohydroxy-benzoic acid, which is well-known signalling molecule playing role in local and systemic acquired resistance against pathogens as well as in acclimation to certain abiotic stressors (Raskin 1992a; Horváth et al. 2007; Szalai et al. 2013; Pál et al. 2013).

SA can be synthesized through phenylalanine pathway. Phenylalanine is converted into cinnamic acid (CA) by phenylalanine ammonia lyase (PAL). PAL is a key regulator of the phenylpropanoid pathway and is induced under a variety of biotic and abiotic stress conditions (Chen et al. 2009). SA can be synthesized from CA *via* two different routes: it can be either hydroxylated to form *ortho*-hydroxycinnamic acid (*o*HCA) followed by oxidation of the side chain or, alternatively, the side chain of cinnamic acid can be initially oxidized to give benzoic acid as an immediate precursor, which is then hydroxylated in the *ortho* position (Alibert et al. 1972; Hayat et al. 2010). The phenylalanine pathway also serves as a rich source of metabolites in plants, such as flavonoids, coumarins and also lignans (Fraser and Chappel 2011). Different pathways of SA biosynthesis are active in different plant species. Feeding of ^{14}C-labeled phenylalanine and cinnamate to young *Primula acaulis* and American wintergreen leaf segments indicated that SA was formed via *o*HCA (El-Basyouni 1964). In sunflower, potato and pea, isotope feeding indicated that SA was formed from benzoate,

which was synthesized by cinnamate chain shortening reactions most likely through a β-oxidation process analogous to fatty acid β-oxidation (Klambt 1962).

Recent studies showed that the phenylalanine pathway cannot account for all SA in plant cells, suggesting that there is another main route for SA biosynthesis taking place in the chloroplasts, where SA is synthesized from chorismate *via* isochorismate in processes catalysed by the isochorismate synthase (ICS) and isochorismate pyruvate lyase enzymes, respectively (Wildermuth et al. 2001; Wildermuth 2006). The role of shikimate pathway in SA synthesis of young pea plants was also demonstrated (Szalai et al. 2011). This shikimic acid pathway is also a link between metabolism of carbohydrates and biosynthesis of aromatic compounds like phenylalanine, tyrosine and tryptophan (Herrman and Weaver 1999).

SA was shown to be tightly linked to further metabolic alterations, affecting redox homeostasis or photosynthetic performance in plants (Mateo et al. 2006). Exogenous applications of SA affect various physiological, biochemical and molecular processes in plants, including antioxidant system (Horváth et al. 2007; Saruhan et al. 2012). It regulates the activities of various antioxidant enzymes such as, peroxidases, superoxide dismutases, catalases etc., which are the major components of induced plant defence against biotic and abiotic stresses (Vicent and Plasencia 2011). Even more, SA has regulatory effect on glutathione homeostasis (Kusumi et al. 2006). SA regulates the components of its own signalling pathway, being involved also in cross-talk with other pathways mediating plant resistance. SA could be utilized for the induction of plant defence system that enables the plant to withstand many biotic and abiotic stresses (War et al. 2011). Photosynthesis is one of the main targets of environmental stressors. The present review is focused on the effects of SA on the photosynthetic processes.

Effects of exogenous SA on photosynthetic processes under optimal environmental conditions

SA as a positive plant growth regulator

The effects of exogenously applied SA on plant physiological processes under optimal environmental conditions are controversial. Several studies suggest that SA may positively affect the germination or plant growth in various plant species. For example, enhanced germination and seedling growth were recorded in wheat, when the grains were subjected to pre-sowing seed-soaking treatment in SA (Shakirova 2007). Similarly, aqueous solutions of SA, applied as a spray to the shoots of soybean also significantly increased the growth of shoots and especially roots. However, this treatment had no significant effect on photosynthetic rate (Gutiérrez-Coronado *et al.* 1998). In contrast to this other authors reported a significant increase in net photosynthesis (P_N) in Indian mustard (*Brassica juncea* L. Czern & Coss), maize or soybean plants previously sprayed with an optimum concentration (10^{-5} M) of SA (Fariduddin et al. 2003; Khan et al. 2003), while higher concentrations of SA had an inhibitory effect. The increase in photosynthetic rates after application of SA was not always accompanied with increased stomatal conductance levels or transpiration rates; however, the intercellular CO_2 concentrations of plants sprayed with SA were generally lower than the control plants. This suggests that the increases in photosynthetic rates following spray applications of certain phenolic compounds such as SA could be the result of increased enzyme activity related to CO_2 uptake at the chloroplast level, rather than simple increases in stomatal opening (Khan et al. 2003). However, the exact mechanism of the photosynthesis enhancement is still unclear.

Both soaking of seeds or foliar application of SA can also be fruitful in increasing the pigment contents, as it was shown in wheat (Hayat et al. 2005) or in rapeseed (*Brassica napus* L.) (Ghai et al. 2002). However, the positive effect of SA on photosynthetic activity is not always due to an increased chlorophyll level. In cowpea (*Vigna unguiculata*) plants SA treatment increased or decreased chlorophyll content, depending on the genotype (Chandra and Bhatt 1998) or the applied concentration, as 0.001-10μM SA increased, while 1mM SA decreased both chlorophyll and carotenoid contents in the cotyledons of sunflower plants (Cag et al. 2009).

SA as a stress factor

Several studies also show that SA may serve as a stressor as well, which among others, negatively affects the photosynthetic processes, especially above a threshold concentration. The effective concentration may highly depend on the plant species, the way of the application, the duration of the treatment, and the environmental conditions. For example, addition of 0.5 mM SA to the hydroponic solution of young maize plants - the same concentration as that provided protection against low temperature-induced damage in young maize (Janda et al. 1999) or could alleviate the damaging effect of paraquate in barley (Ananieva et al. 2002) - decreased the net photosynthesis under both light-limited and light-saturated conditions together with a decrease in the stomatal conductance (g_s) and transpiration rate. Intercellular CO_2 concentration (C_i) was higher in the dark and lower in the light in the SA-treated plants than in the untreated control maize plants (Janda et al. 1999), respectively. Similarly, while short-term treatment of barley seedlings with SA (for minutes up to 2 hours) did not affect the rate of photosynthesis, leaf or root growth; the chlorophyll and protein contents and the maximal rate of photosynthesis were also reduced by a long-term

treatment with SA together with an increase in both the CO_2 compensation point and stomatal resistance (Pancheva et al. 1996). Increasing SA concentrations also resulted in a significant decrease in the maximum and effective quantum yields of PSII photochemistry (F_v/F_m and Φ_{PSII}, respectively) and in the photochemical quenching parameter in guard cells of tomato plants. In the presence of low SA concentration (10^{-7} or 10^{-4} M), the chloroplasts of guard cells sustained a higher electron transport rate than in the presence of 10^{-3} M, suggesting that the SA-induced inhibition of guard cell photosynthesis may affect stomatal closure at high SA concentrations (Poór and Tari 2012). Application of SA to Arabidopsis leaves also rapidly induced stomatal closure and photoinhibition that ended up in photodamage (Mateo et al. 2004). It must also be mentioned that not only SA, but certain related compounds, such as acetylsalicylate (aspirin), or a possible precursor benzoic acid showed similar effects. These compounds, besides affecting the gas exchange rates, may also influence the processes related to the photosynthetic electron transport via enhancing the non-photochemical fluorescence quenching (NPQ) mechanisms (Janda et al. 2000).

Transients of the development of NPQ during continuous illumination were also modified in the SA-infiltrated tobacco leaves, indicating that SA influenced the build-up of the transmembrane pH gradient (Janda et al. 2012). The slower development of NPQ after exposing the leaves to light in SA-infiltrated tobacco leaves may also be due to a slower rate of zeaxanthin formation (Bilger and Björkman 1991). Light response curves of NPQ showed that SA treated leaves had limited capacities of regulated energy dissipation, especially at higher light intensities. The highly reduced rate of electron transport occurring under high light conditions may also explain the reduced NPQ in plants treated with SA at high concentration (Janda et al. 2012).

In barley plants the ribulose-l,5-bisphosphate carboxylase activity decreased while the activity of phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) increased with increasing

SA concentrations (Pancheva et al. 1996). The observed inhibition of photosynthesis and the activity of Rubisco by SA are mainly caused by a decrease in the Rubisco content (Pancheva et al. 1998). However, short-term treatment of barley seedlings with SA also decreased the levels of chlorophyll, CO_2 assimilation and transpiration rates without changes in the Rubisco activity (Ananieva et al. 2002). These results suggested that SA may cause a decrease in photosynthetic activity under normal growth conditions; however, the influences on the Rubisco activity seem to be secondary, long-term effects. Furthermore, in a recent proteomic study with maize plants, the SA responsive protein, Rubisco large subunit, which was represented by two protein spots interestingly showed opposite expression patterns at protein level: one was up-regulated while the other was down-regulated. One hypothesis concerning this phenomenon is that the same protein in maize leaves may have different isoforms and have different roles under phytohormone stress (Wu et al. 2013). Pyruvate orthophosphate dikinase, which plays a controlling role in the PEP-regeneration phase of the C4 photosynthetic pathway, was also down-regulated by SA treatment in maize leaves (Wu et al. 2013).

While growing of wheat plants in solution containing a wide range of SA concentrations (0.05-1 mM) stimulated the electron flow associated with Photosystem II, the Photosystem I-associated electron transport activity was only stimulated at low concentrations of SA, and at higher concentrations it was drastically attenuated (Sahu et al. 2002). Thylakoids isolated from the leaves of wheat seedlings grown with high concentrations showed a substantial reduction in uncoupler (NH_4Cl)-mediated stimulation in electron flow. In addition, they failed to support ADP-dependent stimulation of electron transport activity and induced a significant reduction in ATPase activity. Incubation of isolated thylakoids with SA, however, had no effect on thylakoid photofunction, indicating no direct effect of SA on photoelectron transport activity. Furthermore, high concentrations of SA specifically reduce

the thylakoid cytochrome f_{554} level. The results suggest that SA, depending on its concentration, imparts differential effects on the photofunction of thylakoids. A low concentration of SA may favour photosynthetic activity while the high concentration induces its drastic attenuation partly due to the decline in cytochrome f_{554} (Sahu et al. 2002).

When 7-day old barley seedlings were supplied with SA through the transpiration stream for 24 h no marked changes in photosynthetic reactions were observed (Maslenkova et al. 2009). However, exposure of barley plants to SA imposed through the root medium for a period of 7 days have a marked effect on the charge recombination of both intact leaves and isolated chloroplast, characterised by thermoluminescence (TL) measurements. These results suggested that SA action was on thylakoid membrane level. SA treatment decreased the number of PSII centres and disturbed the redox cycling of the rest operative water-oxidising centres. Reduction of Hill-reaction activity and oxygen flash yields is in accordance with the changes in membrane permeability and malondialdehyde content indicating that long-term SA treatment leads to the development of oxidative stress symptoms. It has also been shown that prolonged treatment with SA resulted in a great number of quantitative alterations in polypeptide composition (Metodiev et al. 2002). The changes in polypeptides belonging to PSII and especially the extrinsic polypeptides of water-oxidizing complex may be important. Thylakoid membranes isolated from SA-treated plants showed strong depletion of 33 kDa extrinsic polypeptide. This protein is important for the functional integrity of the manganese cluster, which catalyses photosynthetic water-splitting; and may also bind GTP pointing to additional functions e.g., participation in signal transduction associated with the thylakoid membrane, possibly connected to the GTP-dependent turn-over of the D1 protein (Spetea et al. 2004). The decrease in its quantity may affect the conformation of PSII reaction centre and the redox properties of some other redox components of electron transport chain. The observed alterations in chloroplast membrane composition as well as the changes in

membrane granal structure during prolonged SA application to the root medium of barley seedlings (Uzunova and Popova 2000) may be a prerequisite for the inhibition of PSII functional activity. SA-induced changes in PSII structure and functions suggested a picture, which is starting to outline that a mechanism other than stomata function can also be involved in the inhibition of photosynthesis (Maslenkova et al. 2009).

Infiltration of SA in tobacco leaves also reduced the CO_2 incorporation and stomatal conductivity. In these plants PSII electron transport was more sensitive to SA than PSI. Direct probing of PSII charge separation and stabilization measured with TL, however, showed that these events were much less affected in isolated thylakoid membranes than in intact leaves, supporting the idea that the effect of SA on PSII is indirect (Janda et al. 2012). In addition to changes in energy partitioning, over-reduction of PSII may also lead to the formation of reactive oxygen species (ROS). Detecting elevated amounts of H_2O_2 in SA treated leaves supports this possibility (Janda et al. 2012). Accumulation of H_2O_2 in the cells also suggests that SA also has a secondary, indirect effect, which is light independent (Fig. 1).

So exogenous SA treatment may either increase or decrease the photosynthetic activity of plants. This may also depend on the species, although the fact that similar effects can also be observed in various genetically different plant types (C_3 or C_4 types of photosynthesis; monocots or dicots; etc.), suggests that other factors, such as concentration, duration of the treatment, way of the application (spraying, adding to hydroponic or soaking of seeds), or environmental condition (temperature, light) may have more important influence than the species specificity itself.

Effects of exogenous SA on photosynthetic processes under stress conditions

Several stressors may directly or indirectly severely affect the photosynthetic processes (Majláth et al. 2012; Ashraf and Harris 2013). It has been well documented that exogenous SA may alleviate the damaging effects of various stress factors, and this protection can also be manifested in higher photosynthetic capacity (Horváth et al. 2007, Hayat et al. 2010, Radwan and Soltan 2012, Sasheva et al. 2013, Tirani et al. 2013). For example, pre-soaking of seeds in 0.5 mM SA solution for 6 h before sowing substantially decreased harmful effects of cadmium on CO_2 fixation in maize (Krantev et al. 2008) or pea (Popova et al. 2009). The activities of carboxylating enzymes PEPC and especially Rubisco were also negatively affected by Cd treatment. However, pre-treatment of maize plants with SA before exposure to Cd alleviated the inhibitory effect of Cd on Rubisco, and led to a nearly 2-fold increase in PEPC activity compared with untreated plants (Krantev et al. 2008).

The illumination of unfrozen dark adapted leaves from pea plants with one short saturating flash induces a main TL peak at 35 °C, the B-band, which is the result of charge recombination between the S2 state of the water splitting system and the secondary quinone acceptor Q_B, together with a hardly distinguishable shoulder at temperature about 40 °C. The latter is referred as AG (afterglow)-band, and is due to a heat-induced electron transfer from stroma reductants to PSII centres initially in the non-recombining state S2/3 Q_B, which is enabled to emit AG luminescence as soon as Q_B is reduced by back-electron transfer (Ducruet 2003). A damping of period-four oscillation of the B-band intensity, according to exciting flash number was also observed at high concentration of Cd together with a decrease in the AG/B ratio. This tendency was much better expressed in the case of TL induction by short far-red illumination of dark adapted pea leaves. This kind of illumination excites mainly PSI but a part of the energy can be absorbed in PSII antenna leading to charge separation and the induction of TL B-band. Short term application of SA to the pea seeds did not exert changes in the investigated TL parameters. However, pre-treatment with SA before the imposition of

high concentration of heavy metal Cd had a stabilizing effect on photochemical reactions, as indicated by some restoration of AG/B ratio and B-band oscillation pattern (Popova et al. 2009).

The photosynthetic thylakoid has the highest level of lipid unsaturation of any membrane. Cd caused a shift in fatty acids composition, resulting in a lower degree of their unsaturation and an increase in saturated fatty acids in sunflower leaves, whereas SA improved them. SA particularly increased the percentage of linolenic acid and lowered that of palmitic acid by the same proportion. These results suggest that SA could be used as a potential growth regulator and a stabilizer of protection of cadmium-induced oxidative stress to improve plant resistance to Cd stress (Moradkhani et al. 2012).

SA also counteracted salt stress-induced growth inhibition (Singh and Gautam 2013) and enhanced the net photosynthetic rate (P_N) in wheat (Arfan et al. 2007) and in tomato plants (Gémes et al. 2008, Poór et al. 2011). Foliar spraying with SA caused increase in g_s, but it did not change the sub-stomatal CO_2 during salt stress in sunflower (Noreen and Ashraf 2008). In cucumber foliar spraying of 0.5-2.5 mM SA before low temperature and light intensity treatment reversed the stress-induced decrease of the leaf P_N, g_s, transpiration rate, Φ_{PSII}, and F_v/F_m, and increase of the C_i (Liu et al. 2009). It is suggested that the stomatal limitations were not the controlling factors for photosynthesis; and SA may affect certain metabolic factors in carbon uptake or fixation including Rubisco enzyme concentration and activity, and/or photosynthetic carbon reduction cycle. Similar conclusion was found during salt stress, as the improvement in growth and grain yield due to SA application was associated with improved photosynthetic capacity, which was not due to stomatal limitations, but was associated with metabolic factors, other than photosynthetic pigments and leaf carotenoids (Arfan et al. 2007).

It was also reported that exogenous SA could protect the photosynthetic apparatus against strong light-induced photodamage in *Satsuma mandarin* leaves. This protective effect was related to electron transport rate and quantum yield of PSII, and relative contents of D1 protein and Deg1 protease induced by exogenous SA under combined high temperature and strong light conditions (Qiu et al. 2011). SA pre-treatment in wheat could not only increase the protein kinase activity, retard the degradation of D1 protein during heat and high light stress, but also accelerated the recovery of D1 protein level when the stress was removed. Correspondingly, the maximum and the actual photochemical efficiency of PSII, electron transfer rate and P_N in SA-treated leaves were higher than that in leaves of control under both stress and non-stress conditions. These results suggest that SA pre-treatment could significantly alleviate damages of heat and high light stress on PSII in wheat leaves, and accelerate restoration of photosynthetic function (Zhao et al. 2011).

SA did not influence P_N of leaves of grapevine plants under normal conditions, while SA could alleviate declines in P_N and Rubisco activition state.SA and did not alter negative changes in PSII parameters (donor side, acceptor side and reaction centre Q_A) under heat stress. Following heat treatment, the recovery of P_N in SA-treated leaves was accelerated compared to the control, and donor and acceptor parameters of PS II in SA-treated leaves recovered to normal levels more rapidly than in the controls. Before heat stress, SA did not affect level of HSP21, but the HSP21 immune signal increased in both SA-treated and control leaves during heat stress. During the recovery, HSP21 levels remained high through the end of the experiment in the SA-treated leaves, but decreased in controls. Based on these results it was concluded that SA pre-treatment alleviated the heat stress induced decrease in P_N mainly through maintaining higher Rubisco activation state, and it accelerated the recovery of P_N mainly through effects on PSII function. These effects of SA may be related in part to enhanced levels of HSP21 (Wang et al. 2010).

Endogenous SA and photosynthesis

Level of SA varies in the range of several orders of magnitude in the different plant species. Under optimal environmental conditions usually very low concentrations (in the range of a few 10-100 ng/g FW) can be detected from the leaves of Arabidopsis, tobacco, maize or wheat plant (Meuwly and Métraux 1993, Mateo et al. 2006, Janda et al. 2007, Szalai and Janda 2009). Certain environmental stimuli, such as pathogen infection, heavy metal contamination, or the use of direct oxidative agent may dramatically, in some cases even in 10-50-fold increase it up to the range of μg/g FW (Raskin 1992b, Enyedi 1999; Pál et al. 2005). However, in some species, such as rice, the basic acid level in the leaves is usually as high as 30-40 μg/g FW (Silverman et al. 1995, Pál et al. 2014).

Plants with reduced SA levels

Arabidopsis mutant *sid2* defective in SA synthesis or *NahG* plants expressing a bacterial salicylate hydroxylase can be characterised with decreased levels of SA, they usually display impaired pathogen defence and slightly impaired PSII operating efficiency and enhanced thermal energy dissipation under low light conditions without impairment of growth or reduced levels of starch. However, these mutants seemed to be unable to increase photosynthetic electron transport efficiency or glutathione levels to the same extent as wild type plants during exposure to high light suggesting that appropriate levels of SA and/or H_2O_2 are required proper light acclimation responses. To compensate their lower ability in light acclimation, these plants need other acclimation processes, for example elevated level of anthocyanin level. These results strongly support the hypothesis that controlled SA levels are

important for optimal photosynthetic performance and growth under different light conditions (Mateo et al. 2006). SA-degrading transgenic *NahG* plants targeted either to the cytoplasm or chloroplasts displayed normal state transition, therefore, the role of SA in state transition could be excluded (Gawronski et al. 2013).

Plants have evolved several mechanisms to get rid of this excess energy in photosynthetic membranes, some of which involve isoprenoid compounds. α-Tocopherol and carotenoids represent a conserved mechanism of photoprotection, while other isoprenoids, such as monoterpenes, represent an additional or alternative photoprotection mechanism (Penuelas and Munné-Bosch 2005). α-Tocopherol and carotenoids display antioxidant activity and therefore contribute to maintenance of thylakoid membrane structure and function under stress. In another experiment while no substantial differences in RWC were observed between wild type and SA-deficient *NahG* transgenic lines of *Arabidopsis* plants, SA deficiency suppressed the water stress-induced loss of chlorophylls and carotenoids observed in the wild type. In addition, accumulations of monoterpenes and α-tocopherol were also suppressed in *NahG* plants both under irrigated and water stress conditions, although the effects were more apparent under stress when as this was more severe. Results suggest that SA affects isoprenoid accumulation in leaves especially under water stress conditions. It is assumes that SA may also be involved in accelerating water-stress induced chlorophyll loss, which may be probably linked with an accelerated senescence under stress (Munné-Bosch et al. 2007).

Plants with elevated SA levels

Arabidopsis mutants with high SA content, such as *cpr1-1, cpr5-1, cpr6-1* or *dnd1-1* were usually characterised by dwarf phenotype (Mateo et al. 2006). However, this dwarfism was

less pronounced when these plants were grown in high light, suggesting that the inhibitory effect of SA on growth was partly overcome at higher light intensities. On the other hand, mutants with low foliar SA content were impaired in acclimation to transient exposure to high light and thus predisposed to oxidative stress. In rice plants, which have two orders of magnitude higher levels of SA than the majority of plant species and appear to be insensitive to exogenous SA treatment, SA also plays an important role to modulate redox balance and protect plants from oxidative stress (Yang et al. 2004). These observations implied an essential role of SA in the light acclimation processes (Mateo et al. 2006).

The increased or reduced levels of SA may impair the photosynthetic processes in a different way (Mateo et al. 2006). The *dnd1-1* (defence no death) mutation in *Arabidopsis* plants affects a cyclic nucleotide gated cation channel (AtCNGC2) and induces constitutive SA-dependent pathogen resistance in the absence of hypersensitive cell death (Yu et al. 1998, Clough et al. 2000). Cpr5 is a membrane protein of unknown function and mutations in *Cpr5* have pleiotropic effects on the regulation of cell death, cell elongation, and trichome development (Kirik et al. 2001, Yoshida et al. 2002). These mutants could be characterised with constitutively high levels of SA, and showed reduced stomatal conductance, higher dark respiration and lower maximum CO_2 assimilation rates, decreased maximum efficiency and quantum yield of PSII, and increased NPQ representing the thermal dissipation of absorbed light energy under low light conditions. However, accumulation of photoassimilates as starch strongly decreased in *dnd1-1*, but not *cpr5-1*, possibly indicating changes in assimilate partitioning (Mateo et al. 2006). Although mutants with high foliar SA content had lower maximum and operating efficiency of PSII than the wild-type, they were able to increase glutathione content and to improve the operating efficiency of PSII during acclimatory responses to high light stress. The inhibitory effects of foliar SA, glutathione, and H_2O_2

accumulation observed under low light conditions seem to be less pronounced at high light when the metabolism is adjusted to higher levels of these compounds.

Photosynthetic electron transport characteristics, H_2O_2, glutathione biosynthesis and changes in leaf water status have been implicated in the signalling pathway regulating the expression of *Apx2* encoding a cytosolic ascorbate peroxidase gene. Besides its antioxidant role ascorbate may also play a role in the regulation of photosynthetic electron transport processes (Tóth et al. 2013). A lower threshold for *Apx2* induction was consistently observed in mutants with elevated SA levels (Mateo et al. 2006).

In contrast to *Arabidopsis*, where SA overproduction leads to dwarfism, transgenic *Populus tremula × alba* expressing a bacterial SA synthase hyperaccumulated SA and SA conjugates without negative growth consequences (Xue et al. 2013). In the absence of stress, endogenously elevated SA elicited widespread metabolic and transcriptional changes that resembled those of wild-type plants exposed to oxidative stress-promoting heat treatments. Potential signalling and oxidative stress markers azelaic and gluconic acids as well as antioxidant chlorogenic acids were strongly co-regulated with SA, while soluble sugars and other phenylpropanoids were inversely correlated. Photosynthetic responses to heat were attenuated in SA-overproducing plants. Network analysis identified potential drivers of SA-mediated transcriptome rewiring, including receptor-like kinases and WRKY transcription factors. Orthologs of *Arabidopsis* SA signalling components NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1) and thioredoxins were not represented. However, all members of the expanded *Populus* nucleoredoxin-1 family exhibited increased expression and increased network connectivity in SA-overproducing *Populus*, suggesting a previously unknown role in SA-mediated redox regulation. The SA response in *Populus* involved a reprogramming of carbon uptake and partitioning during stress that is compatible

with constitutive chemical defence and sustained growth, contrasting with the SA response in Arabidopsis, which is transient and compromises growth if sustained.

Stress-induced SA levels

In addition to the application of exogenous SA being reported to provide protection against several types of stressors, unfavourable environmental conditions have also been shown to increase the endogenous SA level in plants (Horváth et al. 2007, Szalai and Janda 2009, Pál et al. 2013). It has long been suggested that different stressors, such as UV light, ozone fumigation or virus infection can activate a common signal transduction pathway that leads to SA accumulation and increased stress resistance (Yalpani et al. 1994). SA accumulation in *Nicotiana benthamiana* depended on ICS activity: total SA levels increased more in wild-type plants than in plants with silenced *ICS* expression after UV irradiation treatment (Catinot et al. 2008).

Similarly to other stressors, low temperature could also induce endogenous SA accumulation in cucumber leaves, and this accumulation could be prevented by spraying with an inhibitor of SA biosynthesis, paclobutrazol. Low temperature resulted in a reduction in maximum and effective photochemical efficiency of PSII, and electron transport rate. Spraying with paclobutrazol enhanced the reduction in PSII efficiency. Low temperature stress led to a decreased allocation of light absorbed by PSII antenna to the photochemical reaction and an increased allocation of excessive energy, and paclobutrazol-treatment caused a greater allocation of light to dissipation as excessive energy. The expression of genes encoding the Rubisco small subunit and carbonic anhydrase was down-regulated by low temperature stress, and the reduction was greater in paclobutrazol-treated seedlings. These results suggested that low temperature-induced SA accumulation was required for

maintenance of photosynthetic efficiency and carbon assimilation capacity, and thereby protected cucumber seedlings against low temperature-induced damages (Li et al. 2013).

Furthermore, exogenous SA may also enhance the plant's own *de novo* SA synthesis, as it was illustrated by the changes in free and bound SA levels in pea plants growing from seeds pre-treated with SA before sowing (Szalai et al. 2011). Pre-soaking the seeds in SA also increased the activities of some antioxidant enzymes, namely ascorbate peroxidase and guaiacol peroxidase and the level of *o*HCA, which may also affect the tolerance to environmental stressors (Fig. 2).

Conclusion

As a stress-related signalling compound, SA may directly or indirectly affect various physiological processes, including photosynthesis. Its mode of action depends greatly on several factors, such as the plant species, the environmental conditions (light, temperature, etc.) and the concentration. When it is applied exogenously, it may provide protection against various types of stressors; however, above a threshold concentration it may also cause damage. Although several phenolic compounds have been shown to have a direct effect on the photosynthetic electron transport chain, the majority of results suggest that the effect of SA on the photosynthetic machinery is indirect, originating from its influence on stomatal conductivity. ROS also play a decisive role in both the upstream and downstream processes of SA signalling. Recent results strongly suggest that controlled SA levels are important in plants for optimal photosynthetic performance and for acclimation to changing environmental stimuli.

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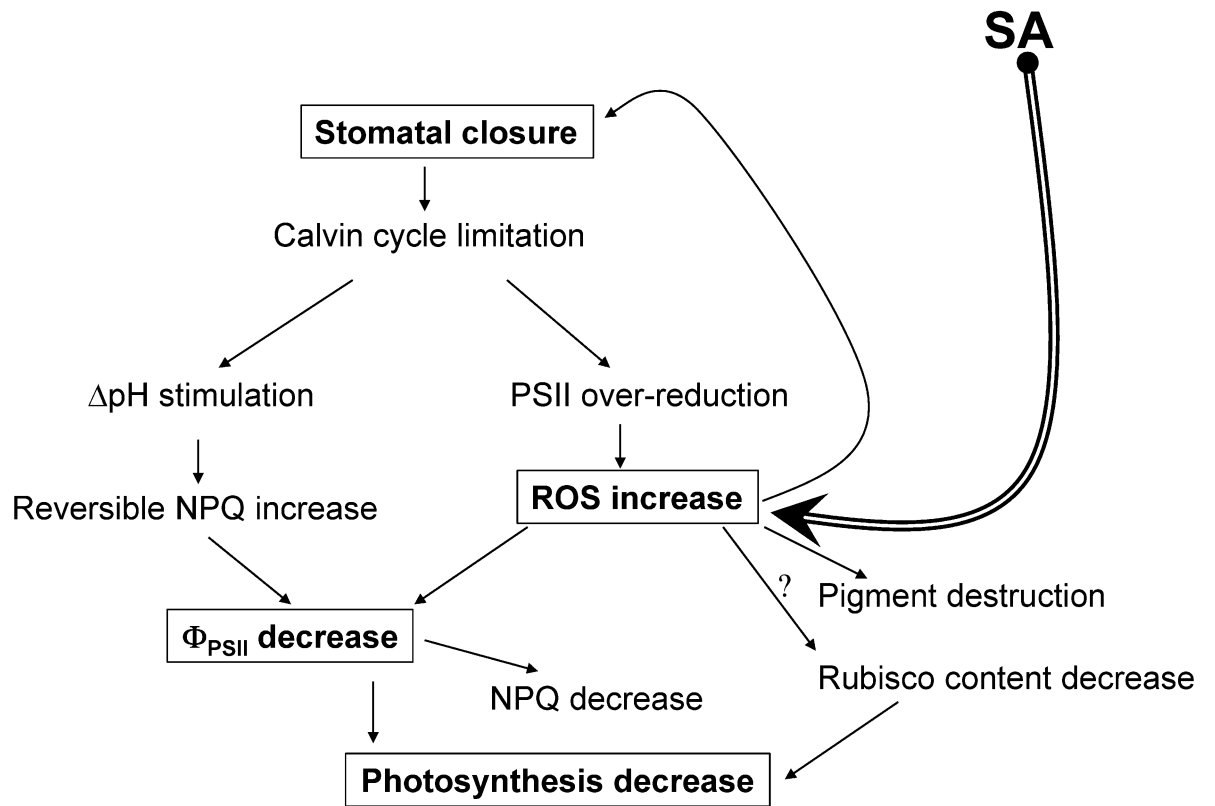
Fig. 1 Schematic representation of the effect of SA on photosynthetic processes. Details in the text.
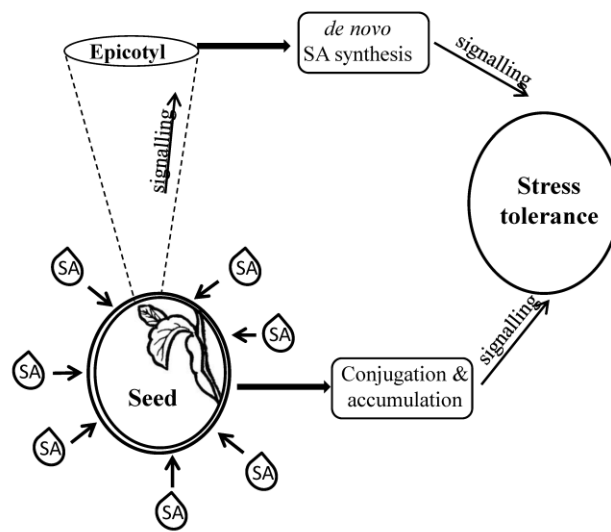
Fig. 2 Exogenous SA may induce *de novo* SA synthesis in plants (Szalai et al. 2011).