RESEARCH ARTICLE

Resistance to cyanide by salicylate pretreatment in *Salix babylonica* L.

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ABSTRACT: Cyanide is uncontrollably produced in some industrial operations and has lethal effects on humans and the environment. Since removing processes of cyanide are complicated and costly, the phytoremediation has recently been extensively considered. To achieve an increased cyanide resistance, which is needed for an efficient phytoremediation of the mediums with high concentrations of cyanide, the effects of salicylate as a phytohormone were studied. Cuttings of *Salix babylonica*, as a model plant, were used in a completely randomized design with three replicates in hydroponics using a modified Hoagland nutrient solution. Plants were pretreated with sulfosalicylate (0, 5, 10 and 15 mg L^{-1}) for 21 days and then were treated with toxic concentration of cyanide (9 mg $CN^{-} L^{-1}$); some of the physiological indices which show cyanide toxicity/resistance were measured. Favorable responses to salicylate toward the increase in resistance to cyanide were concentration dependent which were observed at 10 mg L^{-1} sulfosalicylate and it was accompanied with an increase in superoxide dismutase activity and reducing the capacity of root extract. Dehydrogenase activity and electrolyte leakage from roots were decreased relating to control plants. It also prevented the cyanide inhibitory effect on oxygen consumption. The observed effects could be attributed to redox status and alteration of production and scavenging of reactive oxygen species by salicylate and cyanide. The results indicated that a proper concentration of salicylate could be used as a cyanide resistance stimulator in willows.

KEYWORDS: redox status, respiration, ROS, SOD, tolerance.

ABBREVIATIONS: AOX: alternative oxidase, CN^{-}: cyanide, ROS: reactive oxygen species, IV complex: IV complex in mitochondrial respiratory chain, SOD: superoxide dismutase.

INTRODUCTION

Cyanide (CN^{-}) is an anion composed of a carbon triply bonded to a nitrogen atom. This anion is one of the most lethal chemicals and highly used in different industries such as gold, lead and zinc extraction and also in the production of nylon and plastics. In addition to the regular usages of CN^{-}, it is a byproduct of some industrial processes in which high concentrations of carbon and nitrogen are put together in a hot and reducing condition, such as chemical manufacturing, iron and steel making, petroleum refining, and aluminum smelting. The free CN^{-}, whatever its sources, finally enters into the wastewater or releases to the environment which, if possible, must be refined. Different methods are usually used for CN^{-} detoxification including naturally scavenging of CN^{-}, alkaline chlorination and oxidation using hydrogen peroxide. Management of CN^{-} using chemical processes is costly, complicated and usually some new harmful byproducts are produced. Indeed, the phytoremediation of CN^{-} has recently been paid attention to. Phytoremediation is the use of vascular plants, algae, and fungi to metabolize or sequester contaminants, or to induce contaminant breakdown by micro-organisms in soil (McCutcheon and Schnoor 2003).

Although CN^{-} is a highly toxic substance and has a high potential to prevent different metabolic pathways, there are almost 2,000 species known as cyanogenic plants which produce high amounts of CN^{-} (mainly as glucosides) for

defense purpose (Halkier and Møller 1990). In addition, in all vascular plants and in some of the algae and fungi, it is produced as a byproduct during the synthesis of ethylene. Since plants, similarly to the other organisms, are sensitive to CN^-, they must hold different strategies for scavenging the produced CN^-.

CN^- affects general metabolism of the cells through blocking of electron transport chain in mitochondria and chloroplasts, decreasing oxygen consumption and inhibiting the activity of some of the metal containing enzymes such as Cu and Zn isoform of superoxide dismutase (Cu/Zn SOD). The change in the cells' energy status and the malfunction of cellular protective enzymes could induce disintegration in membranes and electrolyte leakage from the cells. Plant mitochondria contains a CN^- resistant electron transport chain called alternative oxidase (AOX) by which they are able to tolerate increased concentrations of CN^-. In addition, different metabolic pathways, such as cyanoalanine pathway (Tittle et al. 1990), make it possible to metabolize CN^- by plants. Indeed, the plants could have significant potential for the phytoremediation of CN^-.

Willow (*Salix* spp.) has been considered a promising model plant in the studies of CN^- phytoremediation (Ebbs et al. 2006). Desirable properties of willows for phytoremediation are: fast growth and accumulation of high biomass, high abilities to uptake water and chemicals from the environment, and high water evaporation rates. Bushey et al. (2006a) showed that the destination of CN^- after uptake by *Salix eriocephala* is the assimilation into some of the cellular metabolites, as its evaporation from the plant is negligible. Different studies have been performed with the aim of modeling a practical CN^- phytoremediation (Bushey et al. 2006a, 2006b). For instance, uptake and translocation of CN^- in free and other forms have been observed in different plant species such as *Hordeum vulgar*, *Sorghum bicolor*, *Eucalyptus* spp., *Avena sativa* and *Salix eriocephala* (Ebbs et al. 2003, 2008, Samiotakis and Ebbs 2004). It has also shown that some of the physiological conditions, such as nitrogen nutrition status and environmental stresses like water deficiency, induce the assimilation pathways of CN^- (Ebbs et al. 2010, Machingura and Ebbs 2010, Machingura et al. 2013).

The increase of the AOX ability and metabolizing pathways have special importance in the phytoremediation of CN^-. Salicylate as a plant hormone strongly stimulates AOX (Van der Straeten et al. 1995). In addition, it stimulates reactive oxygen species (ROS) production and scavenging systems in plants (Hayat et al. 2007). In this study, the effects of salicylate on the resistance of *Salix babylonica* to CN^- were investigated. Some of the potential or known responses of plants to salicylate and CN^- were tested to conclude about the responsible mechanisms of acquired resistance. The metabolic and other response pathways overlapped in the responses to both salicylate and CN^- are targets of this study. We also measured the responses simultaneously in order to attain a more comprehensive view on these interactions.

MATERIAL AND METHODS

Preparing the cuttings and culture medium: Cuttings with 150 to 200 mm long were prepared from semi-woody two-year old branches of a *Salix babylonica* tree in late spring of 2011. Cuttings were placed in perlite by half of the length from base, watered with tap water and kept in a growth chamber with a light regime of 16:8 (light:dark) constant temperature of 24°C. The rooting of the cuttings occurred after 25 d. A hydroponic culture was used for the treatment of plants. The cuttings were then transferred to the greenhouse in pots containing 10 L of a modified Hoagland solution pH 5.8, containing 0.5 mM $CaNO_3$, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.5 mM KNO_3, 0.005 mM FeEDDHA (Ferric ethylenediamine–di-2-hydroxyphenylacetate), 0.01 mM H_3BO_3, 2 µM $MnCl_2$, 0.2 µM $ZnSO_4$, 0.2 µM $CuSO_4$ and 0.1 µM Na_2MoO_4.

The solutions were aerated periodically every 3 h and exchanged every 10 d. Evaporation was compensated daily by adding distilled water to the solutions. The plants were kept in this situation until complete adaptation to the greenhouse conditions. Then, the experiments were performed in the greenhouse during the late spring and summer. The range of temperature during the day was 25–36°C and in the night was 22–25°C.

Although the pH of the Hoagland solution had been adjusted to 5.8, after the addition of CN^- the solution pH was adjusted to 7.8 to reduce evaporation of CN^-, which occurs at lower pH. Since CN^- complexes with different metals and the complexes are not easily absorbed by plants, the concentration of culture solutions were reduced 1/10 to diminish complex formation after the treatment of plants with CN^-.

Pre-treatment by salicylate and treatment of plants by cyanide: Two plants were transferred into pots containing 1.5 L culture solution before the treatments. Plants with even weights were selected and the total weight of each plant was 120±10 g. The plants were pre-treated with different concentrations of sulfosalicylate including 0, 5, 10 and 15 mg L^{-1} for 2 weeks. The treatment with CN^- was performed by using NaCN at a toxic concentration of 9 mg CN^- for 24 h. The toxicity threshold of CN^- concentration was determined by submitting the plants to different concentrations of CN^- at

0, 3, 5, 9, 15 and 20 mg $CN^- L^{-1}$. After the treatments, different parameters including oxygen consumption by roots, leakage of electrolytes from roots, reducing capacity of root extract, dehydrogenase and superoxide dismutase activities in roots were measured.

Measurement of oxygen consumption: The roots were cut (1 g FW, fresh weight), washed twice with distilled water and then transferred into test tubes containing 45 mL of distilled water. Dissolved oxygen was measured every 2 h using an oxygen meter (WTW with an electrode model Cellox 325, Germany). The oxygen meter was calibrated before use based on the device manual.

Measurement of the electrolyte leakage: After harvesting, 1 g FW of root from each treatment was subjected to desorption using an ice cooled desorption solution containing 5 mM $CaSO_4$ and 5 mM Na_2EDTA, pH 5.8, for 10 min, and then washed twice with bidistilled water. The roots were then transferred into test tubes containing 45 mL of bidistilled water and shaken very gently at 50 rpm. The electrical conductivity (EC) of the water was measured every 2 h until 6 h, using an EC-meter (Elmetron, Poland).

Reducing capacity test: The reducing capacity of the extract was measured based on the ferric reducing antioxidant power (FRAP) test (Benzie and Strain 1999). A section of root (1 g FW) was grinded with liquid nitrogen using an ice cooled mortar and pestle. Then, 3 mL of 50 mM phosphate buffer, pH 6, were added and after more grinding the extracts were centrifuged at 12,000 x *g* at 4°C for 10 min. In all steps, the temperature was kept below 4°C. The reducing capacity was performed using of TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine). At low pH, reducing Fe^{3+} to Fe^{2+} by reducing agents of the extract causes an intense blue color after the formation of TPTZ-Fe^{2+} complex, with an absorption peak at 593 nm. A standard curve was obtained using different concentrations of $FeSO_4$ which was linear between 10 and 100 µM $FeSO_4$.

Measurement of dehydrogenase activity: Dehydrogenase activity was measured according to Kittock and Law (1968) by using 20 g FW of root submerged in 1 mL of 1% TTC (2,3,5 three-phenyltetrazolium-chloride) solution for 2 h. The roots were then submerged into 3 mL of methyl cellosolve (2-methoxyethanol) solvent and gently shaken for 6 h until the roots became white. The solution was then separated and centrifuged at 10,000 x *g* for 10 min. Finally, the absorption was measured at 480 nm and the results were expressed as percentage of the control plants.

Measurement of the superoxide dismutase activity: The activity of SOD (1.15.1.1) was measured according to Giannopolitis and Ries (1997). The reaction solution contained 50 mM buffer phosphate, pH 7.8, 13 mM methionine, 75 µM NBT (ρ-nitrobluetetrazolium chloride), 2 µM riboflavin and 0.1 mM EDTA. The reaction was started by adding 100 µL of root extract (from the preparation above mentioned) to 1,000 µL of reaction solution, mixed and put under florescence light with the intensity of 30 µmol photon $m^{-2} s^{-1}$ for 15 min. Using dark samples as blank, the absorption was measured at 560 nm. Based on definition, half of the reaction of converting of NBT (yellowish) to Formazan (gray-bluish) is defined as a unit of SOD activity.

Measurement of cyanide in medium: A colorimetric method was used to measure CN^- in medium (Goulden et al. 1972) by adding 1 mL of 1 M NaOH and 1 mL of 0.4% of chloramine T to 5 mL of each sample. The mixture was kept for 2 min and then 1 mL of pyridinebarbituric acid was added to each sample. The mixture was kept for 5 min during which time a purple color appeared. Light absorption at 578 nm was measured. Using a standard curve (at the range concentration of 0.02–0.2 mg $CN^- L^{-1}$), the concentration of CN^- in medium was determined.

Statistical analyses: The experiments were performed in a complete randomized design. To determine the statistically significant differences between the means, multiple comparisons were performed by one way ANOVA and Tukey's HSD test. The Statistical Package for the Social Sciences (SPSS) software (version 16 for Windows; SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The reported data are the means for 3 replicates ± SD.

RESULTS

Resistance was studied from two viewpoints. Firstly, from the occurrence of toxicity symptoms in plants and second from the duration between the treatment and the occurrence of the symptoms. In high concentrations of CN^-, the roots turned brownish and the shoots were dried without necrosis, destruction of chlorophyll or shedding of leaves. In moderate concentrations of CN^-, growth depression of the apical meristems, shedding of leaves and yellowing of leaf interveins were observed. At non-toxic concentrations, none of the symptoms were observed. Acute symptoms appeared at the concentrations of 15 and 20 mg $CN^- L^{-1}$ after 24 h. Moderate symptoms of toxicity at 9 mg L^{-1} were observed after 48 h. At the concentrations of 3 and 5 mg $CN^- L^{-1}$, no toxicity was observed. The results also showed that pretreatment of

plants with sulfosalicylate reduced the symptoms at 9 mg L^{-1} CN^-. After 24 h, some moderate symptoms were observed but they did not persist and the plants returned to normal growth.

Table 1 shows the viability of pretreated plants after being treated by toxic concentration of CN^- (9 mg CN^- L^{-1}). Pretreatment by sulfosalicylate caused statistically significant increase in plant viability after the treatment by lethal concentration of CN^- (9 mg CN^- L^{-1}) in 48 h. Among the different concentrations of sulfosalicylate used, 10 mg L^{-1} showed the highest viability.

The amounts of consumed CN^- by pretreated plants are shown in Figure 1. In all treatments, more than 95% of the added CN^- was absorbed by roots. The highest consumption was observed by control and at 5 mg L^{-1} sulfosalicylate pretreated plants. By increasing the concentration of sulfosalicylate, less uptake of CN^- was observed ($p<0.05$).

Figure 2 shows the oxygen consumption by roots treated with different concentrations of sulfosalicylate. In CN^- treated plants which were not treated with sulfosalicylate, a significant decrease in oxygen consumption was observed ($p<0.01$) while no decrease was observed in sulfosalicylate treated plants.

The treatment with CN^- induced electrolyte leakage from roots ($p<0.05$). In comparison to the control plants with no pretreatment of salicylate, less leakage of electrolytes was observed from the roots that were pretreated with 10 mg L^{-1} sulfosalicylate (Figure 3). Pretreatment by 5 and 15 mg CN^- L^{-1} caused no change or even induced an increase in electrolyte leakage.

The dehydrogenase activity in roots was decreased with CN^- ($p<0.01$). By increasing the concentration of sulfosalicylate from 5 to 10 and 15 mg L^{-1}, a substantial decrease in dehydrogenase activity was observed (Figure 4). The results showed that the SOD activity was significantly increased ($p<0.05$) in CN^- treated plants. Pretreatment of plants by sulfosalicylate also had a more stimulatory effect which was statistically significant at 10 mg CN^- L^{-1} added to the medium ($p<0.05$; Figure 5).

The treatment by CN^- in non-pretreated plants was accompanied by a significant decrease in reducing capacity, while at 15 mg L^{-1} sulfosalicylate no change was observed. At the pretreatments of 5 and 10 mg L^{-1} sulfosalicylate, a significant increase was observed as compared with the control non-pretreated plants ($p<0.05$; Figure 6).

Table 1. Viability of the sulfosalicylate pretreated plants (*Salix babylonica*) treated by 9 mg CN^- L^{-1} in 48 and 72 h. Amounts are expressed as percentage of viable plants

Sulfosalicylate concentration (mg L^{-1})	Viability (%)	
	48 h	72 h
0	50	0
5	100	66.6
10	100	100
15	66.6	50

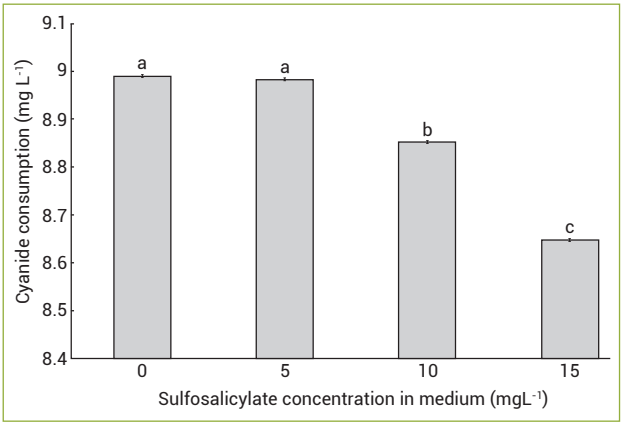

Figure 1. Consumption of cyanide by salicylate pretreated plants during 24 h. Bars represent arithmetic means±SD of n=3 replicate samples, each containing 2 plants. Different letters show statistically significant differences ($p<0.05$) between different concentrations of sulfosalicylate based on ANOVA (Tukey's HSD test).

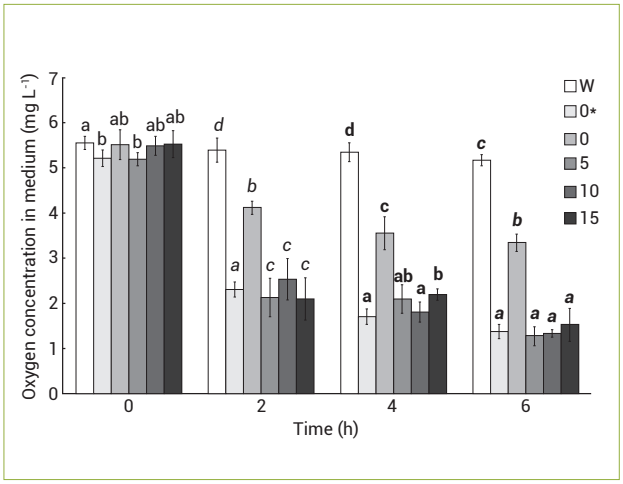

Figure 2. Effect of pretreatment of different concentrations of sulfosalicylate and treatment of toxic concentration of cyanide (9 mg CN^- L^{-1}) on the oxygen consumption by *Salix babylonica* roots. Bars represent arithmetic means±SD of n=3 replicate samples, each containing 2 plants. Different letters in each series of time (normal letters for the time of 0, italicized letters for 2 h, bold letters for 4 h and italicized bold letters for 6 h) show statistically significant differences ($p<0.05$) between different concentrations of sulfosalicylate and control plants based on ANOVA (Tukey's HSD test). W: water without root; 0*: roots without neither pretreatment of sulfosalicylate nor treatment of cyanide; 0, 5, 10 and 15: pretreatment of 0 to 15 mg L^{-1}sulfosalicylate.

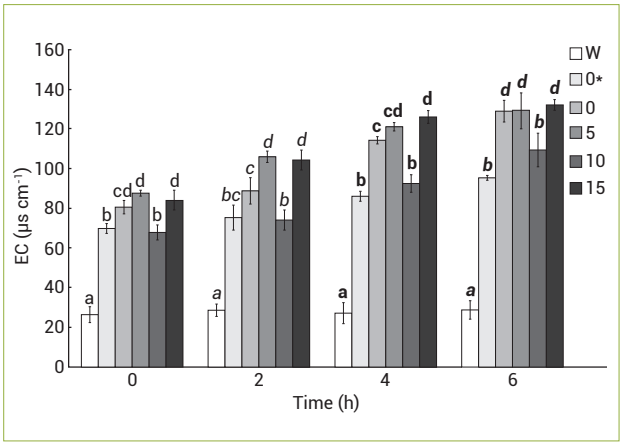

Figure 3. Effect of pretreatment of different concentrations of sulfosalicylate and treatment of toxic concentration of cyanide (9 mg CN^- L^{-1}) on the leakage of electrolytes from *Salix babylonica* roots. Bars represent arithmetic means±SD of n=3 replicate samples, each containing 2 plants. Different letters in each series of time (normal letters for the time of 0, italicized letters for 2 h, bold letters for 4 h and italicized bold letters for 6 h) show statistically significant differences ($p<0.05$) between different concentrations of sulfosalicylate and control plants based on ANOVA (Tukey's HSD test). EC: electrical conductivity; W: water without root; 0*: roots without neither pretreatment of sulfosalicylate nor treatment of cyanide; 0, 5, 10 and 15: pretreatment of 0 to 15 mg L^{-1}sulfosalicylate.

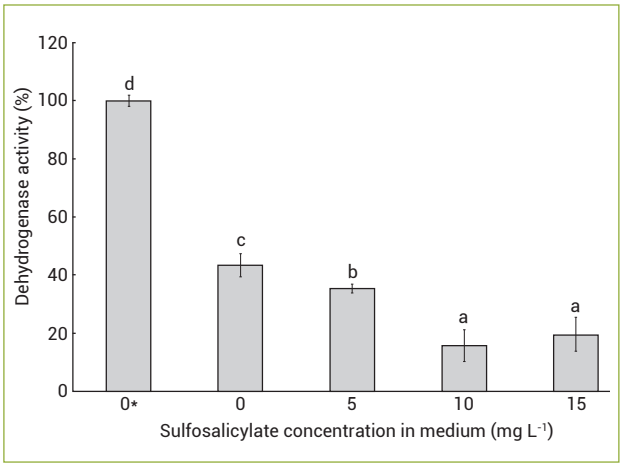

Figure 4. Dehydrogenase activity of the *Salix babylonica* roots pretreated by different concentrations of sulfosalicylate and treated with 9 mg L^{-1} cyanide. Bars represent arithmetic means±SD of n=3 replicate samples, each containing 2 plants. Different letters show statistically significant differences ($p<0.01$) between different concentrations of sulfosalicylate based on ANOVA (Tukey's HSD test). 0*: roots without neither pretreatment of sulfosalicylate nor treatment of cyanide.

DISCUSSION

Plants could be considered as CN^- consumers since, during the metabolism of the ethylene, it is produced with the ratio of 1:1 (Taiz and Zeiger 2002) and the produced CN^- should be metabolized or released from tissues. In studies focusing

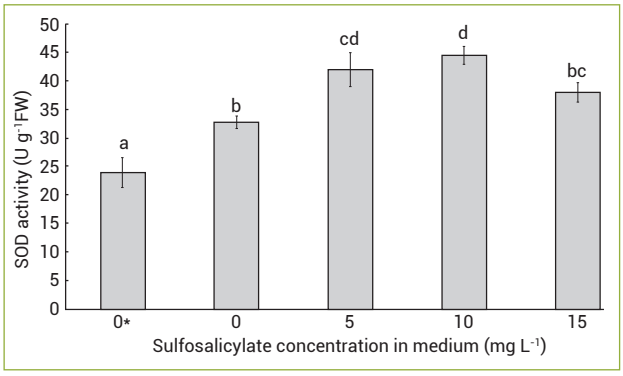

Figure 5. Superoxide dismutase activity of the *Salix babylonica* roots pretreated by different concentrations of sulfosalicylate and treated with 9 mg L^{-1} cyanide. Bars represent arithmetic means±SD of n=3 replicate samples, each containing 2 plants. Different letters show statistically significant differences ($p<0.01$) between different concentrations of sulfoalicylate based on ANOVA (Tukey's HSD test). 0*: roots without neither pretreatment of sulfosalicylate nor treatment of cyanide; SOD: superoxide dismutase.

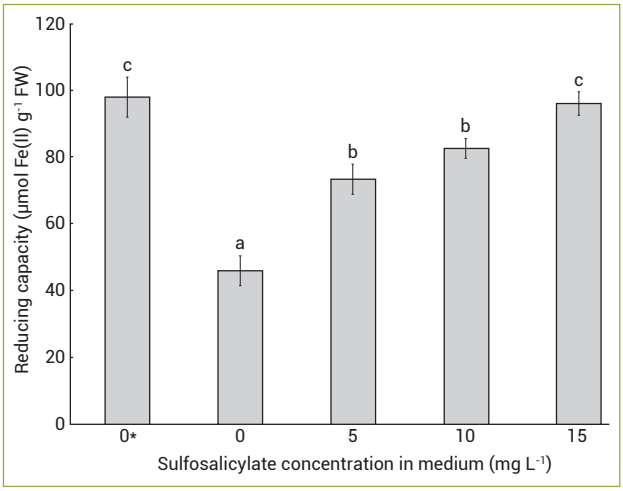

Figure 6. Reducing capacity of the *Salix babylonica* root extracts pretreated by different concentrations of sulfosalicylate and treated with 9 mg L^{-1} cyanide. Bars represent arithmetic means±SD of n=3 replicate samples, each containing 2 plants. Different letters show statistically significant differences ($p<0.01$) between different concentrations of sulfosalicylate based on ANOVA (Tukey's HSD test).

the resistance of plants to the unfavorable chemical agents, an important question is whether the resistant plant is able to metabolize the agent. In other words, do plants convert the toxic agent to a non-toxic form? Some studies have shown that no clear correlation could be found between resistance against the unfavorable agent and its detoxification (Mauricio 2000, Rausher 1992). Resistance is a mechanism that moderate the damage on the plant and tolerance is the ability of a plant to

experience the damage devoid of reduction in the vigor (Baker 1987, Rausher 1992). To achieve this condition, plant metabolism interferes with structure and properties of enzymatic systems and changes the physico-chemical properties of cell membranes and transporters. Resistance to CN^- may be due to the metabolization and destroying of the CN^- and, in plants which have evolved mechanism(s) of ameliorating, the effects of CN^-, the activities of metabolic pathways such as sulfur transferase and cyanoalanine have positive correlation with CN^- resistance (Blumenthal et al. 1968, Castric et al. 1972).

Immediate metabolization of CN^- in different cellular metabolizing pathways prevents CN^- from reaching sensitive sites such as metals in metalloproteins. More active metabolizing pathways means less concentration of CN^- in the cells and more resistance to higher concentrations of external CN^-. Since the activity of different metabolizing pathways strongly is affected by the status of nutrition, energy, hormones and environmental conditions (Goudey et al. 1989; Tittle et al. 1990), resistance to cyanide changes by change in remarked factors.

One of the phytohormones which strongly affects the energy status and respiratory activities of the cells is salicylate (Hayat et al. 2007). This hormone strongly affects electron transport chain in mitochondria. The effect of salicylate on cellular respiration is concentration-dependent (Xie and Chen 1999). However, in the current study, no difference was observed in oxygen consumption at different concentrations of salicylate. It has a dual role in the production of ROS in the cells. If salicylate promotes disturbing in the respiration chain in mitochondria, more ROS are produced (Maxwell et al. 2002). The reason could be a mechanism like CN^- and antimycine A – both inhibit the oxidation of cytochrome c (Chen et al. 1993). On the other hand, salicylate is one of the most important stimulators of CN^- resistant respiratory pathway or AOX pathway (Rhoads and McIntosh 1992). Therefore, salicylate is an inhibitor of electron transport that increases ROS production and also activates AOX, which by consuming reducing agents prevents ROS production. In this study, we observed that the activity of SOD increased at the presence of salicylate. This finding could be due to an increase in the production of ROS by salicylate and CN^- and consequently stimulation of antioxidant system of the cells (such as SOD). CN^- inhibits not only electron transport chain at the IV complex in mitochondrial respiratory chain (IV complex) but also some of the metalloenzymes such as Cu/Zn containing a form of superoxide dismutase (Cu/Zn SOD), which is the most important isoform of SOD in cytosol (Tsang et al. 1991).

In this study, the treatment by CN^- decreased the reducing power of the root extracts, which were not pretreated by salicylate. This result is possibly due to high production of oxidizing agents (such as ROS). The pretreatment of salicylate increased the reducing capacity, but it was not different of the control plants. The effect of salicylate could be due to preventing ROS production.

Enzymatic dehydrogenase activity in the root cells was decreased by CN^-. We hypothesized that the long time pretreatment with salicylate and its inhibitory effect on electron transport chain, which occurs at the site of reduction of ubiquinone (Norman et al. 2004), could reduce the electron transport. By comparing with different studies, Galis and Matsuoka (2007) concluded that several hundreds of genes could be modulated by to salicylic acid including dehydrogenases. Therefore, it is pondered that the redox status of the cells is affected by salicylate by changing the activity of dehydrogenases.

ROS has a central role in maintaining the homeostasis of redox state of cells and the increase or decrease in their concentrations affects the activity of producing or scavenging pathways (Mittler 2002). We also observed that salicylate increased ion leakage from the roots (except at the concentration of 10 mg L^{-1} sulfosalicylate). Ion leakage is a result of high concentrations of ROS in the cells, as it causes lipid oxidation and impairs cellular membranes integrity, whose physiological consequence is ion leakage (Dietz et al. 1999).

It could be hypothesized that salicylate affected the cellular homeostasis by increasing the ROS production, which subsequently increased the activity of SOD and the activation of AOX. Increased activity of AOX prevents the decrease in oxygen consumption at the presence of CN^- and favors the cells with ATP production at low rates. The most important findings of this study are summarized in Table 2.

The results suggest that, to increase resistance of plants against CN^-, a concentration dependent pretreatment of salicylate might be used. In addition, measured resistance parameters will be useful to assess the activity of the CN^- consuming or metabolizing pathways with the salicylate pretreatment since the activity of these pathways is considered as resistance index.

Table 2. Summarized results of the current study and a short description on the most possible reasons of the observed results. The results are a comparison between pretreated (10 mg L^{-1}) or other concentrations (5 and 15 mg L^{-1}) of sulfosalicylate and control plants. Proper concentration of sulfosalicylate was defined based on the occurrence of a noticeable resistance

Measured	Result		Description
	Proper concentration of sulfosalicylate (10 mg L^{-1})	Other used concentrations of sulfosalicylate	
SOD activity	Increase	Equal or increase	Proper concentration showed the most stimulation resulted in appropriate ROS scavenge
Oxygen consumption	Increase	Increase	Possibly due to activation of AOX pathway by salicylate
Electrolyte leakage	Decrease	Equal or increase	A balanced cellular redox status and ROS production/scavenge at proper concentration of salicylate and keeping membrane integrity
Dehydrogenase activity	Decrease	Decrease	Inhibition by cyanide after treatment or hormonal effect of salicylate on gene expression
Reducing capacity	Increase	Increase	Accumulation of reducing agent(s) due to inhibition of electron transport chain
Uptake of cyanide from medium	Decrease	Equal or decrease	In the cases of decrease, still more than 95% of added cyanide into the medium
Resistance to lethal concentration of cyanide	Best result	Less suitable	Salicylate can be used to increase resistance in a concentration dependent mode

SOD: superoxide dismutase; ROS: reactive oxygen species; AOX: alternative oxidase.

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