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# EFFECTS OF SALICYLIC ACID ON THE GROWTH AND PHYSIOLOGICAL CHARACTERISTICS IN *CYCLOCARYA PALIURUS* SEEDLINGS

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**Abstract**

Field studies were conducted to examine the effects of salicylic acid (SA) on the growth and physiological characteristics of *Cyclocarya paliurus* seedlings by spraying the foliage with 0.0 (control), 0.2, 1.0, and 2.0 mM salicylic acid (SA). Proper concentrations of SA improved the relative growth yield of seedling stems and the soluble protein and sugar content of the leaves. It also increased the content of secondary metabolites including triterpenoids, flavonoids, quercetin and kaempferol, mineral elements K, Ca, Mg, Mn, Fe and Cu. Moreover, it stimulated the activities of superoxide dismutase (SOD), peroxidase (POX) and phenylalanine ammonia-lyase (PAL) in the leaves. The effects of SA on these indices were dose dependent. The relative growth of seedling stem diameter and quercetin content increased gradually with an increase in concentration of SA from 0.0-2.0 mM. A concentration of 0.2 mM was optimal to promote content of soluble protein, sugars, Ca, Mg, Mn, Fe, and Cu, and SOD activity and significanty increased by 38.6%, 22.1%, 17.7%, 8.2%, 20.3%, 23.2%, 15.6%, and 52.4%, respectively, as compared with the control (CK). However, the maximal increase in activities of PAL, POX, and content of triterpenoids, kaempferol, and flavonoids was attained at 1.0 mM treatment, which significantly increased by 76.5%, 78.4%, 76.4%, 96.3%, and 107.4%, respectively, compared with CK. Correlation analysis revealed positive relationships between activities of PAL, POX and content of triterpenoids, quercetin, kaempferol, and flavonoids within a certain concentration range of SA. These results suggested that an appropriate concentration (0.2-1.0 mM) of SA was not only effective in the improvement of physiological function of *C. paliurus*, but also increased seedling resistance; additionally, it helped to stimulate the synthesis of medicinal components in leaves.

**Key words:** salicylic acid, *Cyclocarya paliurus*, Superoxide dismutase, Peroxidase

## Introduction

*Cyclocarya paliurus* (Batal) Iljinskaja, is commonly called ''sweet tea tree" because of the flavor of its leaves (Fang *et al*., 2006). These are sole species in its genus and native to China (Fang *et al*., 2006). The leaves of *C. paliurus* have been a source of food for maritime people for a long time (Birari *et al*., 2007). They have also been used for drug formulations in traditional Chinese medicine (TCM) and as an ingredient in functional foods in China (Xie *et al*., 2010a; Fang *et al*., 2011). Many studies have demonstrated that *C. paliurus* possesses a variety of bioactivities, including antihypertensive activity, hypoglycemic activity, enhancement of mental efficiency, anticancer, Anti-HIV-1 and antioxidant activity (Kurihara *et al*., 2003; Xie *et al*., 2006; Xie *et al*., 2008; Xie *et al*., 2010a; Zhang *et al*., 2010; Xie *et al*., 2013a). These beneficial effects have been partly attributed to its variety of chemical components like protein, polysaccharides, triterpenoids, flavonoids, steroids, saponins, phenolic compounds, and minerals (Xie *et al*., 2004; Xie *et al*., 2010b; Fang *et al*., 2011; Li *et al*., 2011; Xie *et al*., 2013b). All of these studies provide the basis for the present rapidly increasing interest in the use of *C. paliurus* as a functional food ingredient and/or as traditional Chinese medicine. However, less attention was paid to develop the silvics and cultivation of *C. paliurus* (Fang *et al*., 2007). Recently, attempts have been made to develop plantations of *C. paliurus* as a functional food or ingredient to be used in traditional Chinese medicine. Fang *et al*. (2011) investigated the genetic and temporal variations in the flavonoid (quercetin, kaempferol, and isoquercitrin) content in leaves of *C. paliurus*. Similarly, Deng *et al*. (2012) explored the effects of environmental and cultural factors on growth and phytochemical content of *C. paliurus*. However, no information is available on the influence of plant growth regulating substances on growth and phytochemical content of *C. paliurus* leaves.

Salicylic acid (SA) is an endogenous plant growth regulator of phenolic nature. It stimulated plant resistance to biotic and abiotic stress by regulating physiological processes (Karlidag *et al*., 2009) *i.e.* germination, plant growth, transpiration rate, stomatal regulation and photosynthesis, ion uptake and transport (Khan *et al*., 2003; Metwally *et al*., 2003; Khodary 2004; He *et al*., 2010). The exogenous application of SA not only provides protection against various stresses, but also improved growth and productivity of plants (Hayat *et al*., 2010; Rivas-San Vicente & Plasencia 2011; Kong *et al*., 2014). A study with growth promoters, SA exploit as priming agent in maize resulted in better emergence index, enhanced number of grains per cob having higher crud protein and ultimately improved grain yield along with elevated harvesting index (Mahboob *et al*., 2015). Kova ´cˇik *et al*. (2009) reported that SA provokes changes in the gene expression related with the biosynthesis and production of phenylpropanoids, such as

those of phenylalanine ammonia-lyase (PAL), resulting in changes in plant metabolome and an increase in the production of antioxidative polyphenols. This could be a promising approach for improving both plant vigor and their content of medicinally valuable compounds (Amoo *et al*., 2012; Hashmi 2012; Lopez-Orenes *et al*., 2013).

The objectives of present study were to investigate the effects of SA on growth and physiological traits as accumulation of key health-promoting flavonoids (quercetin, kaempferol), total triterpenoids, and some minerals in the leaves of *C. paliurus* under field conditions. Also to explore the relationship between these secondary metabolites with related metabolic enzymes such as PAL. These findings would be of great value for efficacy of SA application and establishing optimal cropping strategies to increase the levels of compounds with potential health benefits and promote the growth of *C. paliurus* plants.

## Materials and Methods

**Experimental field site:** The experimental site is located in Lingfeng ecological farm (31°2′ N latitude and 119°18′ E longitude), Jiangsu Province, China. The site is situated in the subtropical monsoon climate area, which has discernible seasons and abundant rainfall. Annual mean precipitation is about 1149.7 mm, with most of the precipitation distributed from June to September, accounting for 60% of the annual precipitation. The sunshine time in January is about 137.6 h, but 229 h in July. The average annual temperature is 17.5°C and the average growing season is 250 frost-free days. The type of soil is yellow-brown soil with an organic matter content of 3%, total nitrogen content of 1.5 g $kg^{-1}$, and available P (phosphorus) and K (potassium) contents of 15.3 and 150 mg $kg^{-1}$, respectively. Soil pH value is about 6.5.

**Plants and experimental design:** The tested materials were 3-year-old *Cyclocarya paliurus* seedlings, and the experiments were conducted from June to August, 2012. The applications of SA consisted of four concentrations of treatment including 0.0, 0.2, 1.0, and 2.0 mM, represented by control (CK), $SA_1$, $SA_2$, and $SA_3$, respectively. The experiment used a randomized complete block design with three replicates. Each treatment consisted of fifteen plants with similar height and stem diameter. The distance between the plants was about 3.0 m. Approximately 300 $cm^3$ of solution per plant was applied to the foliage until the solution dripped from the plant. A few drops of Tween 20 were added to the solution as surfactant. Spraying was done thrice, once every 15 days, and the first treatment was in late June. The samples (leaves) were collected 15 days later after the last treatment for analysis of physiological indices.

**Growth index:** Prior to treatment applications, initial growth measurements were recorded for each plant including seedling height and stem diameter at the base, represented by $H_0$ and $D_0$, respectively. Two months after treatment, height and stem diameter at the base were measured again and were represented by $H_1$ and $D_1$, respectively. The relative growth increments (RGI) of plant height and stem diameter at the base were calculated by the following equation: $RGI_H= (H_1 - H_0)/ H_0$, $RGI_D=( D_1 - D_0)/ D_0$, respectively.

**Physiological index:** Total soluble proteins were measured following the methods of Bradford (1976), and the soluble sugar content was determined by anthrone colorimetry (Zhang & Chen, 2008). Activities of superoxide dismutase (SOD, E.C.1.15.1.1) and guaiacol peroxidase (POX, E.C.1.11.1.7) were measured by the method of Lu (2012). Phenylalanine ammonia-lyase (PAL, E.C.4.3.1.5) activity was determined according to the method of Zhang (2005). Determination of each index contains three replications.

**Quercetin and Kaempferol:** For the extraction of quercetin and kaempferol, 1.0 g of the dry sample was hydrolyzed with 50 mL of petroleum ether for 2 h and then refluxed with methanol for 4 h at 80°C. After the extract had been evaporated to dryness, the residue was refluxed with a mixture of methanol and HCl (the volume ratio of methanol to HCl is 4:1) at 100°C for 0.5 h. The extract was cooled to room temperature and adjusted to 25 $cm^3$ with methanol. Then, the extract was filtered through a 0.45 μm organic phase filter for HPLC analysis.

The analyses of selected compounds were performed on an Agilent Series 2100 HPLC system (Agilent, Palo Alto City, CA, USA), equipped with a quaternary pump solvent management system, auto sampler, and was connected to Agilent Chem Station software. The separation was carried out on an Eclipse XDB-C18 column (250 mm×4.6 mm, 5 μm) at a column temperature of 30°C. One solvent system with two solvents (A, methanol; B, 0.5% phosphoric acid) was used for quercetin and kaempferol determination. The chromatographic separation was performed by isocratic elution of the mobile phase (mixture of solvents A and B (55:45 v/v) that was filtered under vacuum through a 0.45 μm membrane before use) at a flow rate of 1.0 mL/min. Detection was performed at a wavelength of 360 nm. Identification of kaempferol and quercetin isoquercitrin was achieved by comparing their retention times with those of authentic standards. Quercetin and kaempferol were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

**Total triterpenoids:** A 0.5 g powder sample was put into a 10 $cm^3$ centrifuge tube which contained 8 $cm^3$ 65% ethanol (v/v), and was then extracted at 60°C for 30 min in an ultrasonic extractor. Each sample was extracted twice. The extraction solvent was evaporated by vacuum drying at 60°C water bath. The sample solution was decreased twice by the same volume of petroleum ether, then extracted by saturated butanol for total triterpenoid determination according to the method of Xu *et al*. (2007). The sample solution was put into a glass-stopped tube, and solvent was volatilized in a water bath. A 0.3 $cm^3$ vanillic aldehyde solution (50 g/L in glacial acetic acid) and 0.6 $cm^3$ perchloric acid were added successively. The reaction mixture was heated at 80°C for 10 min in a water bath, then cooled down quickly, and diluted with 3.0 $cm^3$ glacial acetic acid. Finally, the

absorbency was determined at 550 nm. Total triterpenoid content was calculated by calibration curve using ursolic acid (Shanghai Auto Biotech Co., Ltd., HPLC purity ≥98%) as the standard.

**Total flavonoids:** The total flavonoid content was determined by using a colorimetric method (Li e*t al*., 2006). In brief, 1.0 g of a sample was placed in a Soxhlet extractor and refluxed with 70% ethanol for 4 h at 80°C. The extract was evaporated to dryness in a rotary vacuum evaporator at <40°C and then dissolved with 70% ethanol. Exactly 1.0 $cm^3$ of 1% $AlCl_3$ methanol solution was added to a 1 $cm^3$ extract in a 10 $cm^3$ volumetric flask, and the mixture was kept for 15 min at room temperature. After incubation at room temperature for color development, the absorbance at 410 nm was measured. Total flavonoid content was calculated using the standard rutin curve.

**Mineral elements:** Dry powdered samples (0.2 g) were weighed into 100 $cm^3$ glass beakers. One milliliter of 70% $HClO_4$ and 9 $cm^3$ of 98% $H_2SO_4$ were added to the samples and the reaction was incubated overnight. The following morning, the beakers were carefully heated until clear solutions were obtained. The residue was filtered, and the solutions were precisely transferred to 100 $cm^3$ plastic standard flasks, moreover the volume was made up with deionized water. The element concentrations in all samples were measured by using an Optima 4300DV Model inductively coupled plasma-optic emission spectrometry (ICP-OES).

**Statistical analysis:** Data were expressed as means ± standard deviations (S.D.) and analyzed by the SPSS 17.0 statistical software (*SPSS* Inc., Chicago, IL, USA). The results were taken from at least three independent experiments performed in triplicate. The significance of differences between the treatments and control mean values was determined by Duncan's multiple range tests at a significant level of 0.05. Pearson's correlation coefficient was used to study the relationships among the variables.

## Results

The results (Fig. 1) shows that a certain concentration of SA ($SA_1$, $SA_2$) had no obvious influence on the relative growth increments of seedling height in *Cyclocarya paliurus*, but high concentration SA ($SA_3$) significantly ($p$<0.05) decreased the relative growth increments of seedling height, compared to the CK (Fig. 1A).

The relative growth increments of the seedling ground diameter increased gradually with an increase in the concentration of SA. $SA_2$ and $SA_3$ treatments were both significantly higher ($p$<0.05), and increased by 35.8% and 61.4%, respectively, compared with CK (Fig. 1B). These results suggest that suitable concentration of SA ($SA_2$, $SA_3$) have no adverse effect on growth of *Cyclocarya paliurus* seedlings, moreover, accelerate thickening growth.

The effects of SA on some physiological indices in the leaves of *C. paliurus* seedlings are shown in Table 1. All indices presented a similar trend of the initial increase followed by a decrease in response to increasing SA concentration from 0.0 to 2.0 mM. Appropriate concentrations of SA ($SA_1$, $SA_2$) effectively improved the contents of soluble protein and sugar, and stimulated the activities of PAL, SOD, and POX. $SA_1$ treatment significantly improved the contents of soluble protein, sugar and SOD activity, which increased by 38.6%, 22.1%, and 52.4% respectively ($p$<0.05) compared with CK. $SA_2$ treatment was the most effective to improve the PAL and POX activity which resulted in an increase by 76.5% and 78.4% respectively ($p$<0.05) over CK. However, the stimulatory effect of high concentration of SA was weakened and was even negative. Therefore, the stimulatory effects of SA on these physiological indices were dose dependent.

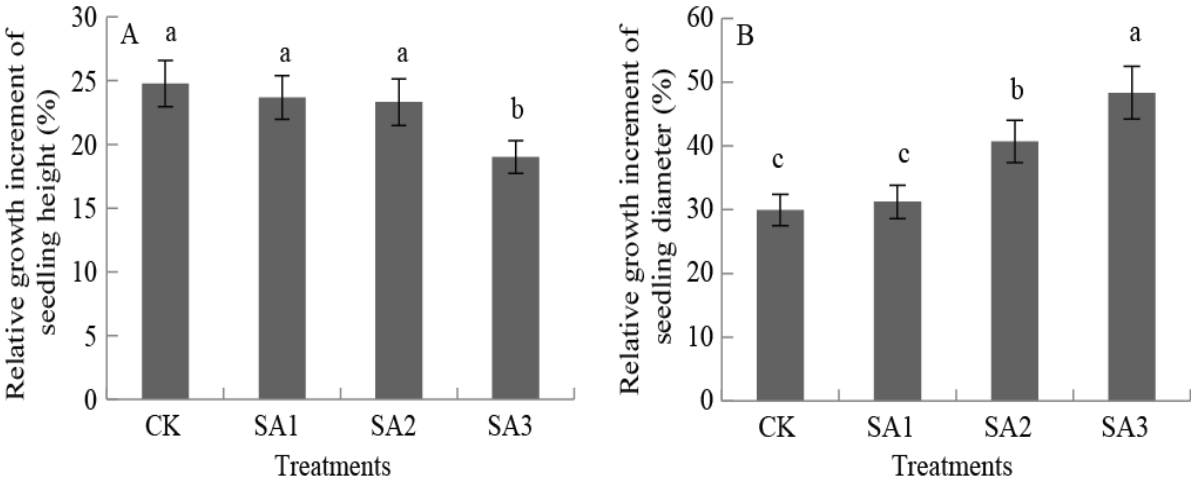


Fig. 1. Effects of different concentrations of SA on the relative growth increments of height **(A)** and ground diameter **(B)** in *C. paliurus* seedlings. *Bars* represent SD (n = 15). Different lowercase letters indicate a significant difference at 0.05 level between different treatments. CK, $SA_1$, $SA_2$, and $SA_3$ represent 0.0, 0.2, 1.0, and 2.0 mM SA treatments, respectively.

**Table 1. Effects of different concentrations of SA on the content of soluble protein, sugar, and the activities of PAL, SOD, and POX in the leaves of *Cyclocarya paliurus* seedlings.**

| Treatments | Soluble protein (mg $g^{-1}$ FW) | Soluble sugar (mg $g^{-1}$ FW) | PAL activity (U $g^{-1}$ FW) | SOD activity (U $g^{-1}$ FW) | POX activity (U $g^{-1}$ FW) |
|---|---|---|---|---|---|
| CK | 15.04 ± 1.23b | 2.67 ± 0.21b | 29.98 ± 2.06c | 1600.00 ± 138.11b | 555.45 ± 46.56b |
| $SA_1$ | 20.85 ± 1.92a | 3.26 ± 0.23a | 48.00 ± 3.45ab | 2437.68 ± 217.12a | 903.31 ± 67.47a |
| $SA_2$ | 17.75 ± 1.62a | 3.03 ± 0.22ab | 52.92 ± 4.24a | 1637.46 ± 146.34b | 991.12 ± 74.32a |
| $SA_3$ | 13.32 ± 0.88b | 2.71 ± 0.15b | 43.58 ± 2.96b | 1230.80 ± 112.32c | 515.36 ± 42.73b |

*Bars* represent SD (n = 6). Different lowercase letters within a column indicate a significant difference at 0.05 level among different treatments. CK, $SA_1$, $SA_2$, and $SA_3$ represent 0.0, 0.2, 1.0, and 2.0 mM SA treatments, respectively

**Table 2. Effects of different concentrations of SA on content of K, Ca, Mg, Mn, Fe, and Cu in the leaves of *Cyclocarya paliurus* seedlings.**

| Treatments | K (mg $kg^{-1}$ DW) | Ca (mg $kg^{-1}$ DW) | Mg (mg $kg^{-1}$ DW) | Mn (mg $kg^{-1}$ DW) | Fe (mg $kg^{-1}$ DW) | Cu (mg $kg^{-1}$ DW) |
|---|---|---|---|---|---|---|
| CK | 2162.5 ± 136.1a | 1585.7 ± 105.2b | 625.3 ± 42.5ab | 383.4 ± 20.2b | 315.2 ± 18.9b | 4.5 ± 0.3b |
| $SA_1$ | 1978.1 ± 122.2a | 1866.3 ± 138.5a | 676.6 ± 48.2a | 461.1 ± 25.6a | 388.4 ± 28.3a | 5.2 ± 0.4a |
| $SA_2$ | 1854.6 ± 110.7b | 1621.3 ± 126.3b | 650.2 ± 54.3ab | 420.3 ± 23.5b | 328.1 ± 21.3b | 4.3 ± 0.3b |
| $SA_3$ | 1483.2 ± 95.4c | 1209.9 ± 106.1c | 585.4 ± 36.6bc | 341.7 ± 20.6c | 252.2 ± 19.2c | 3.5 ± 0.2c |

*Bars* represent SD (n = 3). Different lowercase letters within a column indicate a significant difference at 0.05 level among different treatments. CK, $SA_1$, $SA_2$, and $SA_3$ represent 0.0, 0.2, 1.0, and 2.0 mM SA treatments, respectively

**Table 3. Correlation analysis between metabolic enzyme activities and secondary metabolites. The numbers outside parentheses represent values calculated when the SA concentrations were 0.0-2.0 mM while the numbers in the parentheses represent values calculated under the SA concentrations of 0.0-1.0 mM.**

| Parameters | Triterpenoid content | Quercetin content | Kaempferol content | Flavonoid content |
|---|---|---|---|---|
| SOD activity | 0.502 (0.153) | -0.496 (0.151) | 0.020 (0.014) | -0.314 (-0.056) |
| POX activity | 0.986 (0.976) | -0.138 (0.976) | 0.741 (0.937) | 0.378 (0.910) |
| PAL activity | 0.759 (0.979) | 0.506 (0.979) | 0.941 (0.942) | 0.828 (0.916) |

The contents of triterpenoids, kaempferol, and flavonoids behaved similarly which increased firstly then decreased with rising concentration of SA. Maximum increase was noted for $SA_2$ treatment, and the contents of triterpenoids, kaempferol, and flavonoids increased by 76.4%, 96.3%, and 107.4%, respectively ($p$<0.05), compared with CK (Figs. 2A, 2C, and 2D).

The content of quercetin increased steadily with an increase in the concentration of SA and all the treatments performed better than CK. $SA_3$ was the optimal concentration to improve the quercetin content, which increased by 314.9% with respect to CK (Fig. 2B).

The effects of SA on content of some mineral elements in the leaves of *Cyclocarya paliurus* seedlings are shown in Table 2. Ca, Mg, Mn, Fe, and Cu contents exhibited similar trend of the initial increase followed by a decrease in response to increasing SA concentration from 0.0 to 2.0 mmol/L. $SA_1$ treatment presented the maximal increase of above elements, and the content of which increased by 17.7%, 8.2%, 20.3%, 23.2%, and 15.6%, respectively ($p$<0.05, except for Mg), compared with CK. While the K content decreased with increasing concentrations of SA, however, $SA_1$ behaved like control. At high concentration SA (such as $SA_3$) significant ($p$<0.05) decrease in contents of these elements was observed over CK. These results indicate that appropriate concentrations of SA ($SA_1$) have a positive influence on the mineral element content of the leaves of *Cyclocarya paliurus* seedlings.

Correlation analysis was done in order to explore the connection among metabolic enzyme activities and secondary metabolites under SA treatments. The results (Table 3) showed that there were strong positive correlations between PAL and POX activities and secondary metabolite content within the SA concentrations of 0.0-1.0 mM. PAL activity also showed a stronger positive correlation with secondary metabolite content within the concentration range of 0.0-2.0 mM. POX activity presented positive correlations with the content of triterpenoids, Kaempferol, and flavonoids within the concentration range of 0.0-2.0 mM. In general, the correlations between SOD activity and secondary metabolite content were not strong.

## Discussion

SA have been used in agricultural production to regulate metabolism, improve resistence to stress, and stimulate the growth of crops (Khan *et al*., 2003; Hayat *et al*., 2010; Hashmi *et al*., 2012); however, relatively less research has been carried out on its application in forestry. Moreover, most studies about its regulation effects on plant secondary metabolism were concentrated on *in vitro* conditions such as suspension cells and tissue culture systems (Shabani *et al*., 2009; Kumar *et al*., 2014). In the present study, the effects of SA on growth and physiological characteristics in *C. paliurus* were investigated under field conditions in order to determine an application basis for seedling production and leaf quality improvement. The results showed that the foliar spray with an appropriate concentration (0.2-1.0 mM) of SA could effectively promote widening growth of stems, increase the content of leaf soluble protein, sugar, and improved physiological characteristics of *C. paliurus* seedlings. The effects of SA on growth and primary metabolism of *C. paliurus* is possibly related to the regulation of photosynthesis. Khan *et al*. (2003) have shown that foliar application of SA increased photosynthetic rates in both corn and soybean and shoot dry mass in soybean plants; the stimulation of photosynthetic rates by SA was due to increases in activity inside the leaf but not due to changes in chlorophyll level. However, chlorophyll and carotenoid levels increased by low concentration of SA in shoots of *Cistus heterophyllus* (Lopez-Orenes *et al*., 2013). The mechanism involved in increased photosynthetic rates by SA is still unclear. SA also increased mineral nutrients uptake by plants, modulated the balance of mineral elements, and to enhance growth and metabolism (Yildirim *et al*., 2008; Kong *et al*., 2014). This result was corroborated by our study in *Cyclocarya paliurus*. A low concentration (0.2-1.0 mmol/L) of SA induced an effective increase of mineral elements content in *C. paliurus* leaves, which was beneficial not only for plant metabolism and photosynthesis, but also improved leaf quality and has nutrition and health benefits for the human diet (Xie *et al*., 2013b).

Many studies have demonstrated that SA could improve stress resistance of the plant (Metwally *et al*., 2003; Khodary *et al*., 2004; He *et al*., 2010; Mahboob *et al*., 2015). Our results showed a significant increase in the activities of antioxidant enzymes SOD and POX in *C. paliurus* leaves in response to an optimal concentration of SA, which could strengthened the ability of scavenging reactive oxygen species (ROS) and improved stress resistance in *C. paliurus* seedlings. SA, as an elicitor, not only activated antioxidant defense systems, but also strongly stimulated the production of secondary metabolites in *Cyclocarya paliurus* based on the performance of triterpenoid, kaempferol, quercetin, and flavonoids under SA treatment. Antioxidant enzymes (SOD and POX) may be involved in accumulation of secondary metabolites, which is widely believed to be a part of the defense and stress responses of plants (Peng *et al*., 2013). Our results showed a similar trend in change in the content of triterpenoid, kaempferol, quercetin, flavonoids, and activities of POX within a certain concentration of SA (0.0-0.1 mM) treatments (Fig. 2, Table 1). However, the role of POD in accumulation of secondary metabolites is still unclear.

The phenylpropanoid metabolic pathway plays a very important role in plant secondary metabolism, which is a main mechanism of flavonoid formation (Lister *et al*., 1996). The key enzyme in the first stage of the phenylpropanoid transition is PAL (Creasy *et al*., 1971). Increasing information suggests that endogenous and exogenous SA induce both gene expression and the enzymatic activity of PAL (Chen *et al*., 2006; Kova´cˇik *et al*., 2009), which, in turn, provokes the accumulation of phenylpropanoids (Bate *et al*., 1994). Our results showed that 0.2–2.0 mmol/L SA clearly enhanced PAL activity, and the effect was in agreement with the observed influence of SA on content of flavonoids (kaempferol, quercetin) and triterpenoids, which are important secondary metabolites of *C. paliurus* leaves and are used as indicators of leaf quality. Results of correlation analysis also showed a well positive correlation between PAL activity and these secondary metabolites under different concentrations of SA treatments (Table 3). Therefore, the accumulation of triterpenoids and flavonoids in *C. paliurus* leaves by SA stimulation is related to activation of PAL activity. However, the detailed mechanism of SA stimulating the accumulation of secondary metabolites and PAL activity needs further investigation. Moreover, because the accumulative effect of secondary metabolites induced by SA is related to development phases of plant organ (Fang & Huang 2013), the optimal phase of SA treatment for improvement of secondary metabolites in *C. paliurus* leaves also needs to be explored.

## Conclusion

Foliar spray with 1.0-2.0 mM SA markedly improved the relative growth yield of seedling stems diameter in *C. paliurus*, 0.2-1.0 mM SA increased the contents of soluble protein, soluble sugar, Ca, Mg, Mn, Fe, Cu, and triterpenoids as well as the activities of SOD, POX and PAL in *C. paliurus* leaves. The contents of quercetin, kaempferol and flavonoids were all significantly promoted by 0.2-2.0 mM SA. In general, the optimal concentration required for primary metabolism was lower than that for secondary metabolism, though the SA optimal concentration requirement for the improvement of each factor was different. SA induced increase in PAL and POX activities could be beneficial for the accumulation of triterpenoids and flavonoids. Our results provide valuable insights for seedling cultivation and improvements in leaf quality associated with medicinal components in *C. paliurus*, and also provide reference for the application of SA in forestry production.

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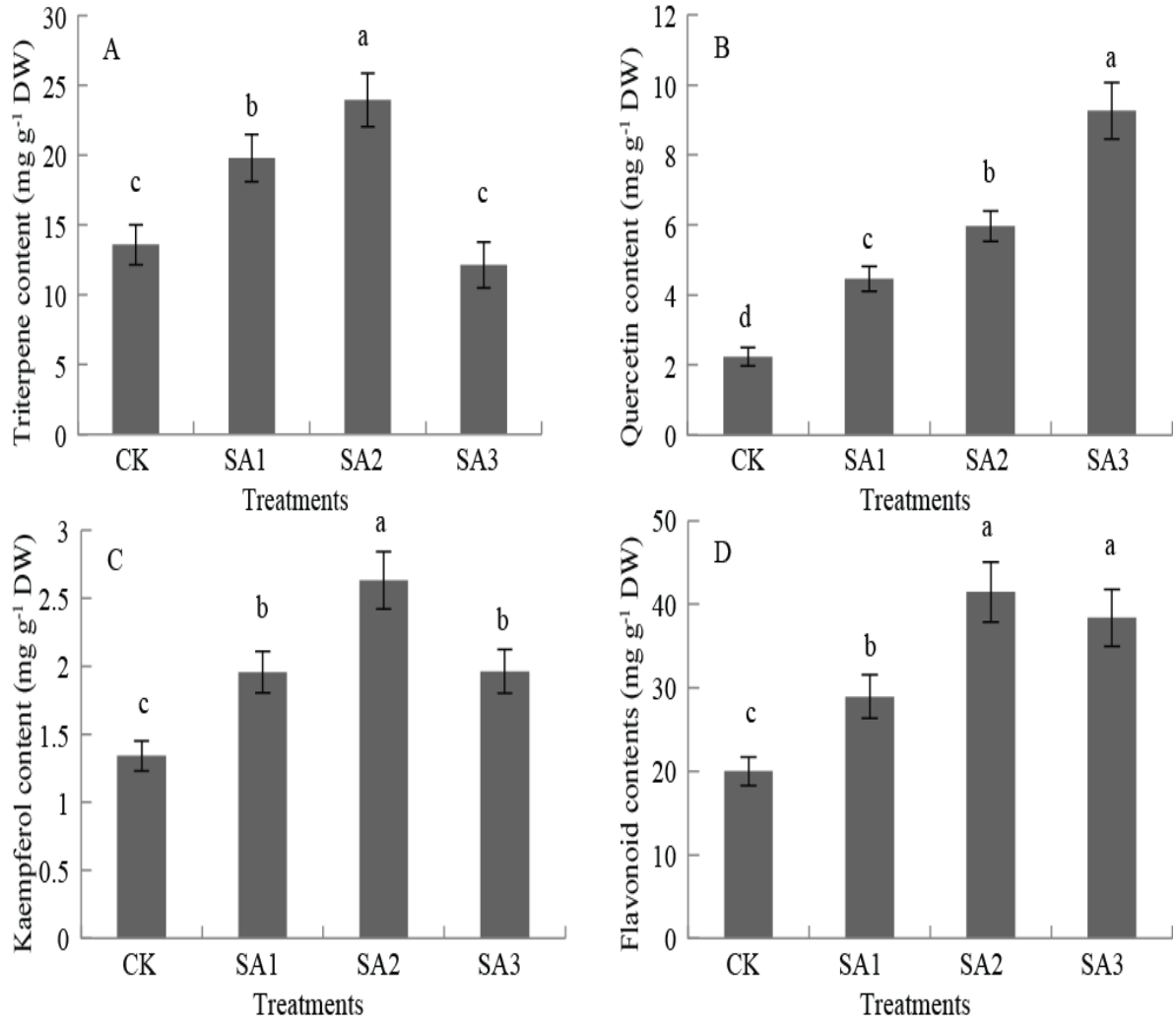


Fig. 2. Effects of different concentrations of SA on contents of total triterpenoids **(A)**, quercetin **(B)** kaempferol **(C)**, and flavonoids **(D)** in the leaves of *C. paliurus*. *Bars* represent SD (n = 6). Different lowercase letters indicate a significant difference at 0.05 level among different treatments. CK, $SA_1$, $SA_2$, and $SA_3$ represent 0.0, 0.2, 1.0, and 2.0 mM SA treatments, respectively.

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