BIOLOGIA PLANTARUM 55 (4): 793-796, 2011

BRIEF COMMUNICATION

Hexanoic acid 2-(diethylamino)ethyl ester enhances chilling tolerance in strawberry seedlings by impact on photosynthesis and antioxidants

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Abstract

Strawberry (*Fragaria ananassa* Duch.) seedlings were pretreated with hexanoic acid 2-(diethylamino)ethyl ester (DA-6) in concentrations of 0, 10, 20 and 40 mg dm^{-3} and then subjected to chilling and rewarming. The effects of applied DA-6 on the generation of reactive oxygen species (O_2^- , H_2O_2), lipid peroxidation, proline accumulation and photosynthesis were evaluated. Pretreatment with DA-6 alleviated the inhibition of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) activities caused by chilling stress thus reducing O_2^- and H_2O_2 production and lipid peroxidation in pretreated plants. DA-6 pretreatment also accelerated accumulation of proline and reduce the decrease in proline content after rewarming. DA-6 pretreatment increases maximum quantum yield of photosystem 2 (F_v/F_m), actual photochemical efficiency of photosystem 2 (Φ_{PS2}), photochemical quenching coefficient (qP) and net photosynthetic rate (P_N) and decreases non-photochemical quenching coefficient (qNP) of the seedlings under chilling stress. DA-6 pretreatment also increased the recovery rate of photosynthesis after rewarming.

Additional key words: ascorbate peroxidase, catalase, chlorophyll fluorescence, *Fragaria ananassa*, net photosynthetic rate, superoxide dismutase.

Low temperature severely affects the growth, yield and quality of plants. Reactive oxygen species (ROS) tends to increase in plants exposed to low temperature (Sun *et al*. 2010). Therefore, plants adopt many efficient defense mechanisms to cope with increased rates of ROS. These mechanisms exploits different antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) and also few non-enzymatic antioxidants such as reduced glutathione (GSH) and ascorbate (AsA) (Asada 1992).

Hexanoic acid 2-(diethylamino) ethyl ester (DA-6) is an artificial tertiary amine. DA-6 is used on a wide range of crops in China either as alone or in combination with different herbicides, fungicides and fertilizers to improve efficacy of crop qualities and production (Liu *et al*. 2005, Zhang *et al*. 2008). DA-6 also plays important roles in modulating the defense response of plants to diverse environmental stresses (Yu *et al*. 2008), including chilling stress (Zhang *et al*. 2001). DA-6 pretreatment can increase photosynthetic rate in wild barley (Zhou *et al*. 2004) and strawberry (Shan *et al*. 2008).

Strawberry can only be grown in sunlight-heated greenhouses during winter and early spring seasons to prevent damages by chilling in northern China. Previous study suggested that DA-6 might improve tolerance of some plants to chilling stress (Shao *et al*. 2007). Therefore this study was carried out to examine the effect of DA-6 on antioxidative system and photosynthesis in strawberry seedlings subjected to chilling stress.

Strawberry (*Fragaria ananassa* Duch.) were grown in

Received 18 September 2010, *accepted* 13 January 2011.

Abbreviations: AsA - ascorbate; APX - ascorbate peroxidase; CAT - catalase; DA-6 - hexanoic acid 2-(diethylamino) ethyl ester; F_v/F_m - variable to maximum chlorophyll fluorescence (maximum quantum yield of photosystem 2); GSH - glutathione; MDA - malondialdehyde; O_2^- - superoxide radical; P_N - net photosynthetic rate; PS 2 - photosystem 2; qNP - non-photochemical quenching coefficient; qP - photochemical quenching coefficient; ROS - reactive oxygen species; SOD - superoxide dismutase; Φ_{PS2} - actual photochemical efficiency of PS 2.

Acknowledgments: This study was supported by the Agriculture Special Fund for the Commonweal Industry of China (No. nyhyzx07-025) and the Qualitative Inspect Special Fund for the Commonweal Industry of China (No. 10-85).

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loamy soil in plastic pots in greenhouse (China Agricultural University, Beijing), at day/night temperature 25/16 °C, 10-h photoperiod, irradiance approximately 600 μmol m^{-2} s^{-1} and air humidity 60 %. The seedlings were watered with 1/2 Hoagland nutrient solution once every two weeks until initiation of chilling stress.

After nine weeks, 100 plants with five fully expanded leaves were chosen and randomly divided into five groups. The non-stressed control plants were kept at 25/16 °C. The other seedlings were treated with water or 10, 20 and 40 mg dm^{-3} DA-6 (supplied by the Crop Chemical Control Research Centre of China Agricultural University) and after 24 h they were subjected to chilling stress in the growth chamber with constant temperature 4 °C for 3 d. Afterwards, plants were transferred to the initial growth chamber for 3 d recovery. The third leaves were harvested for biochemical estimations.

Net photosynthetic rate (P_N) was measured with *Li-6400* portable photosynthesis system (*Li-Cor*, Lincoln, NE, USA). Measurements were made between 9:00 and 10:00 on a sunny day. Six leaves were examined per treatment. Chlorophyll fluorescence was measured with the *FMS-2* fluorescence monitor (*Hansatech*, Norfolk, UK). Before measurement, the leaf samples were kept in darkness for 15 min. Actinic light of 600 μmol m^{-2} s^{-1} and saturating pulse of 8 000 μmol m^{-2} s^{-1} were used. Chl fluorescence parameters were calculated by the method of Schreiber *et al*. (1986) and Genty *et al*. (1989).

The O_2^- production rate was measured following the method of Zhao *et al*. (2008) monitoring the nitrite formation from hydroxylamine in the presence of O_2^-. Measurement of H_2O_2 was practiced according to Alexieva *et al*. (2001). Lipid peroxidation was determined by measuring the content of malondialdehyde (MDA) according to Guo *et al*. (2006). SOD activity was determined by the method of Beauchamp and Fridovich (1971). APX activity was assayed according to Nakano and Asada (1981), recording changes in absorbance at 290 nm due to oxidation of ascorbate. CAT activity was determined by following the reduction of H_2O_2 at 240 nm according to the method of Aebi (1984). The AsA content was assayed by determination of the reduced activity of AsA, according to the method of Law *et al*. (1983). The GSH content was assayed according to the method of Griffith (1980). Proline content was determined using a colorimetric method (Zhu *et al*. 1983).

The results were analyzed with statistical package for social studies (*SPSS v. 16.0*) using one-way analysis of variance (*ANOVA*). Significant differences between the treatment means were separated by using the least significant difference (LSD) test at $P < 0.05$.

ROS accumulation may cause oxidative damage to plant cell membranes, forming toxic products such as MDA (Dhindsa *et al*. 1981). The O_2^-, H_2O_2 and MDA contents significantly increased during chilling stress and slightly decreased after rewarming (Table 1). Pretreatment with DA-6 caused less increases in O_2^- and MDA content caused by chilling stress and higher reduction after rewarming, which depicts that DA-6 could alleviate oxidative damage to leaves of strawberry caused by chilling stress and increase the rate of recovery after rewarming. The possible mechanism could be that DA-6 increase the activity of antioxidative enzymes and the contents of antioxidants.

The activities of SOD, CAT and APX decreased distinctly due to chilling stress and partially restored after rewarming. Seedlings treated with DA-6 not only maintained higher SOD, CAT and APX activity during chilling stress but also promotes restoration of enzyme activities after rewarming (Table 1).

AsA and GSH not only reduce ROS through AsA-GSH cycle, but also directly scavenge O_2^- and reduce H_2O_2 to water (Noctor and Foyer 1998). Chilling treatment sharply increase AsA and GSH contents. DA-6 application further enhanced the AsA and GSH contents (Table 1). Similarly, higher activities of SOD, CAT, APX and higher contents of AsA and GSH were found rather in tolerant than in chilling sensitive genotypes of barley and rice (Dai *et al*. 2009, Zhang *et al*. 2010). Some reports also showed that strawberry subjected to low temperature can increase cold resistance through increase in activity of antioxidative enzymes (Gülen *et al*. 2008, Zhang *et al*. 2008).

Proline is one of the important organic osmolytes in plant cells, and its rapid accumulation has been positively correlated with chilling stress tolerance in plants (Xin and Li 1993**)**. It was found that the proline content in leaves of strawberry increased under chilling stress and decreased after rewarming (Table 1). DA-6 treatment increased the accumulation of proline while retarded its decline after rewarming.

Maximum quantum yield of photosystem (PS) 2 (determined as ratio of variable to maximum fluorescence, F_v/F_m), actual photochemical efficiency of PS 2 (Φ_{PS2}), photochemical quenching coefficient (qP) and net photosynthetic rate (P_N) in the leaves of strawberry seedlings decreased during chilling stress while non-photochemical quenching coefficient (qNP) increased (Table 1). Similar results were obtained by Zhang *et al*. (2010) in rice. The decreases in these parameters were reversed to some extent after rewarming. Pretreatment with DA-6 not only moderated chilling-induced decreases in F_v/F_m, Φ_{PS2}, qP and P_N, but also promoted the recovery of photosynthesis after rewarming.

It is well documented that the function of photosynthetic apparatus is sensitive to high and low temperature stress. PS 2 appears to be preferentially affected by chilling stress (Agati *et al*. 1996, Bertamini *et al*. 2007). The decrease in F_v/F_m, Φ_{PS2}, qP induced by chilling indicated that an important portion of the PS 2 reaction centers was damaged and that DA-6 alleviated

Table 1. Effects of DA-6 on contents of O_2^-, H_2O_2, MDA, activities of SOD, CAT, APX, contents of AsA, GSH and proline, P_N and chlorophyll fluorescence parameters of strawberry after 3 d chilling and after 3 d rewarming. CK - non-stressed plants; T0 - chilling + water; T1 - chilling + 10 mg dm^{-3} DA-6; T2 - chilling + 20 mg dm^{-3} DA-6; T3 - chilling + 30 mg dm^{-3} DA-6. Means ± SE of 3 - 6 replicates in each parameter. The significance of differences between treatments at $P < 0.05$ is represented by different letters (for rewarming in italics).

Parameters	Treatments	CK	T0	T1	T2	T3
O_2^-	chilling	2.15±0.18c	4.32±0.04a	3.22±0.15b	2.80±0.13b	2.88±0.23b
[nmol mg^{-1}(f.m.) min^{-1}]	rewarming	2.19±0.15*c*	3.90±0.14*a*	2.68±0.07*b*	2.44±0.14*bc*	2.58±0.13*bc*
H_2O_2	chilling	2.59±0.09c	4.87±0.07a	4.30±0.18b	3.97±0.10b	4.53±0.19ab
[µmol g^{-1}(f.m.)]	rewarming	2.65±0.18*b*	4.01±0.15*a*	3.70±0.16*a*	3.29±0.10*a*	3.70±0.18*a*
MDA	chilling	14.48±0.59d	28.19±0.41a	24.64±0.88b	21.93±0.70c	24.71±0.20b
[µmol g^{-1}(f.m.)]	rewarming	13.70±0.26*d*	24.19±0.59*a*	23.57±0.53*a*	18.97±0.62*c*	21.82±0.25*b*
SOD	chilling	631.11±20.89a	411.64±4.18c	517.51±5.10b	553.69±24.32b	535.49±8.05b
[U mg^{-1}(f.m.)]	rewarming	622.81±9.63*a*	490.01±8.80*c*	571.71±7.64*b*	622.03±10.21*a*	625.98±18.13*a*
CAT	chilling	653.86±26.07a	468.10±26.11b	650.77±27.59a	542.98±28.26b	484.92±31.63b
[U mg^{-1}(f.m.) min^{-1}]	rewarming	663.30±20.06*a*	533.17±26.59*c*	671.71±16.70*a*	600.12±15.55*b*	611.13±16.18*b*
APX	chilling	32.14±1.15a	20.85±1.13c	24.64±0.88b	26.31±0.72b	26.16±0.67b
[U mg^{-1}(f.m.)]	rewarming	31.37±0.48*a*	25.19±0.40*c*	27.90±0.22*b*	28.93±1.15*ab*	29.37±1.28*a*
AsA	chilling	1.51±0.11c	2.21±0.04b	2.46±0.08ab	2.69±0.08a	2.67±0.08a
[µmol g^{-1}(f.m.)]	rewarming	1.47±0.10*c*	2.08±0.11*b*	2.09±0.02*b*	2.36±0.08*a*	2.18±0.02*ab*
GSH	chilling	27.71±0.99c	36.08±1.87b	40.15±0.95a	42.65±1.04a	41.10±0.89a
[µmol g^{-1}(f.m.)]	rewarming	28.45±0.67*b*	31.61±0.98*b*	35.03±1.54*ab*	37.12±1.13*a*	35.32±1.40*ab*
Proline	chilling	11.91±0.46d	18.00±0.72c	20.46±0.42b	24.34±0.52a	20.79±0.51b
[µg g^{-1}(f.m.)]	rewarming	12.11±0.72*c*	15.16±0.74*b*	16.95±0.55*b*	20.09±0.54*a*	16.14±1.01*b*
P_N	chilling	12.43±0.35a	8.19±0.24d	9.68±0.30c	10.60±0.15b	10.44±0.20bc
[µmol^{-1}(CO_2) $m^{-2}s^{-1}$]	rewarming	12.50±0.60*a*	9.34±0.23*c*	10.78±0.46*b*	12.20±0.25*a*	11.30±0.30*ab*
F_v/F_m	chilling	0.851±0.012a	0.725±0.025c	0.809±0.004b	0.821±0.001a	0.811±0.002ab
	rewarming	0.859±0.002*a*	0.784±0.034*c*	0.816±0.003*b*	0.835±0.011*a*	0.819±0.007*b*
Φ_{PS2}	chilling	0.598±0.039a	0.484±0.076c	0.519±0.025b	0.521±0.167b	0.498±0.118bc
	rewarming	0.554±0.087*a*	0.501±0.074*c*	0.523±0.015*b*	0.536±0.002*a*	0.527±0.074*b*
qP	chilling	0.797±0.070a	0.671±0.120c	0.744±0.022b	0.736±0.260b	0.737±0.05b
	rewarming	0.795±0.125*a*	0.734±0.067*c*	0.751±0.044*b*	0.743±0.15*bc*	0.762±0.128*b*
qNP	chilling	0.612±0.021c	0.706±0.003b	0.648±0.022b	0.686±0.089b	0.760±0.029a
	rewarming	0.609±0.061*c*	0.694±0.045*a*	0.631±0.059*b*	0.633±0.055*b*	0.594±0.101*c*

this damage.

In conclusion, chilling stress injured the strawberry seedlings to a great extent. Treatment of DA-6 can improve the chilling resistance and retard decrease in photosynthesis through modulating antioxidative system.

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