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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 14 (4): 300-304, 2011
ISSN 1028-8880 / DOI: 10.3923/pjbs.2011.300.304

Effects of Salicylic Acid on Growth, Biochemical Constituents in Pepper (*Capsicum annuum* L.) Seedlings

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Abstract: In the present study, the effect of different concentrations of salicylic acid which is an endogenous organic acid in plants and which is commonly cited as a hormone, on the growth and some other parameters of pepper (*Capsicum annuum* L. cv.) seedlings was investigated. The solutions were applied to the roots of seedlings using hydroponic method. In general, 1.5 mM concentration of salicylic acid had a stimulating effect while 5 and 10 mM concentrations had varying degrees of inhibitive effects on the seedlings. Although 0.3 mM SA application produced prominent results in the case of all parameters, the difference was not found statistically significant. The inhibitive effect produced by high SA was found much more dominant than the stimulating effect of low SA concentrations. Thus, it was established that SA had a bidirectional physiological effect on the seedlings in a concentration-dependent manner.

Key words: Salicylic acid, pepper, toxic effect, growth, lipid peroxidation

INTRODUCTION

Salicylic acid which is a secondary plant product, performs important actions in the growth and development processes of plants. It is a potent signaling molecule in plants and is involved in eliciting responses to biotic and abiotic stresses (Krantev *et al*., 2008). These actions include exercising a thermogenic effect (Ansari and Misra, 2007), increasing thermotolerance (Kaur *et al*., 2009), stimulating adventive root formation (Kling and Meyer, 1983), showing herbicidic effect (Shettel and Balke, 1983), reducing leaf shed (Ferrarese *et al*., 1996), providing resistance against pathogens (Alvarez, 2000), regulates ethylene biosynthesis (Huang *et al*., 1993; Srivastava and Dwivedi, 2000) and changing the quality and quantity of proteins (Doares *et al*., 1995). It has been claimed (Ray, 1986) that SA and similar phenolic compounds exercise their effect of providing resistance against different stress factors in plants (Szepesi *et al*., 2005; Yuan and Lin, 2008) by modifying the effects of abscisic acid and gibberellic acid (Apte and Laloraya, 1982) and cytokinins (Ray *et al*., 1983). These observations and reports on many other physiological effects brought about by SA invoked in several researchers the idea that this substance might be a new plant growth regulator (Hayat and Ahmad, 2007). It was purported that SA could exercise such an effect on NRA in the mediation of plant hormones (Schneider and Whitman, 1974). Fariduddin *et al*. (2003) reported increased NR activity due low concentrations of SA while higher concentrations were observed to be inhibitory to NR activity in *Brassica juncea*. A study by Singh *et al*. (2010) showed positive correlation between chlorophyll content and total nitrogen in cucumber cotyledons. Moreover, increase in nitrogen content and chlorophyll content at lower concentrations of SA indicates that this plays a regulatory role during the biosynthesis of active photosynthetic pigments. It is known at present that plants under stress age more rapidly than those under optimum conditions (Vaadia *et al*., 1961). Besides, cytokinins are known to have a delaying effect on proteolytic activity and leaf senescence (Noodén *et al*., 1997). It has been argued that the decline in plastid formation and chlorophyll-carotenoid synthesis in plants exposed to stress results from the accumulation of abscisic acid (Duysen and Freeman, 1976) or the decrease in cytokinins levels (Itai and Ben-Zioni, 1973). In another study carried out on discs of plucked rice leaves, ethylene biosynthesis was found to be inhibited in 2 h following SA administration (Huang *et al*., 1993). In still another study performed in spinach (*Spinacea oleracea* L.) suspension culture, ethylene accumulation inhibited chlorophyll production (Dalton and Street, 1976). Miguel-Chavez *et al*. (2003) observed that stem diameter and height of the plants increased by applying 10^{-10} and 10^{-8} M SA. Similarly, application of 10^{-8} and 10^{-6} M SA increased fresh stem weight, dry stem weight and root length. The commercially available form of salicylic acid is acetylsalicylic acid (ASA) (Mitchell and Broadhead, 1967). SA was observed to reduce leaf area (secondary leaf), root growth, as well as protein and chlorophyll (a+b) amount parallel to an increase in its concentration in barley plants which were developed from barley seeds germinated in SA solutions of varying concentrations and grown in SA-containing milieu (Pancheva *et al*., 1996). Khan *et al*. (2003) found that spraying minute concentrations (10^{-5} mol L^{-1}) of SA and ASA on the leaves led to an increase in the overall photosynthetic yield of soybean and corn. It was reported in the same study that stomatal mobility and transpiration increased while chlorophyll amount remained unchanged. In studies where one-week-old corn and bean seedlings were ASA (50, 250, 1000 ppm) to the root (Canakci and Munzuroglu, 2002) or the leaf (Canakci and Munzuroglu, 2000) caused an increase in fresh weight loss and a decrease in transpiration in high concentrations. It has been reported in a study using discs obtained from primary leaves of one month old bean seedlings that chlorophyll a and b quantity decreased, carotenoid amount remained unaffected while fresh weight loss and protein destruction increased parallel to the increase in ASA concentration (100, 250, 500 ppm) (Canakci, 2003). ASA was found to lead to the closure of stoma pores in high concentrations (Larque-Saavedra, 1978). Environmental stress negatively affects the functions of the plasma membrane which can be measured as the level of lipid peroxidation. The amount of malondialdehyde (MDA) reflects the degree of lipid peroxidation. Study on the effect of SA in relation to oxidative stress indicate that the level of MDA in leaves remained unchanged (Gautam and Singh, 2009), decreased (Krantev *et al*., 2008) or increased (Popova *et al*., 2009) in association with toxic stress in relation to various SA concentrations. Under the circumstances, the objective of the present study is to examine the physiological and some biochemical effects of low and high concentrations of salicylic acid which builds resistance against biotic and abiotic stress in plants, on pepper seedlings in the absence of some other stress factor.

MATERIALS AND METHODS

Plant growth and treatment with salicylic acid solutions: The present study used 30-day-old seedlings produced from pepper (*Capsicum annuum* L. cv. Kapya) seeds were used as the vegetative material. Pepper seeds were obtained from May Seed Group. Seeds were first disinfected with 0.5% sodium hypochloride solution for 5 min and washed with deionised water. Then they were placed on double-folded filter paper wetted with 10 mL deionised water in petri dishes and germinated for 3 days in dark at 24-26°C temperature. Homogeneously germinated seeds were planted in plastic pots which were 25 cm-deep and 20 cm in diameter filled with leaf mould and sand (2:1 v/v). The plants were raised under natural light at 25±5°C and 60% humidity, in 16 h light/8 h dark photoperiod and were watered every other day. Of the 15-day-old seedlings, homogeneous ones were carefully removed from the raising environment and their roots were washed with tap water. These seedlings were placed in the channels of the sponge lids on the mouth of bottles of 150 mL volume and contained 100 mL Hoagland nutrient solution. Nutrient solutions were replaced every two days. When the seedlings were 30-day-old, their roots were treated for 16 h with 0, 0.3, 1.5, 5 and 10 mM salicylic acid test solutions prepared in deionised water using hydroponic method. The seedlings were studied after two days in terms of seedling and leaf length, seedling fresh and dry weight and pigment, protein and MDA content. While dissolving the crystal salicylic acid, 1 mL 95% ethyl alcohol was used (Shettel and Balke, 1983) and solution pH was set to 4.5-5 using 0.1 N NaOH (Larque-Saavedra, 1978). pH measurements were performed with JENWAY 3040 ion analysis using a pH-meter apparatus. Absorbance measurements were carried out using a UVA-093001 Heëlosá spectrophotometer. The growth parameters were determined in 10 seedlings for each group.

Tissue extraction and determination: One gram of fresh leaf obtained from seedlings which were treated with SA from the root was used in each group. For pigment analysis, the leaves were extracted and absorbance of these extracts was read against blind at 645 and 663 nm wavelengths. Quartz tubes of 1 cm^3 volume were employed in the absorbance determinations. Chlorophyll (a+b) amounts were calculated from the absorbance values obtained (Witham *et al*., 1971). In order to establish the protein amount, fresh weights of seedlings to which SA was applied from the root were determined without delay and protein analysis was conducted. Protein extraction was performed according to Larson and Beevers (1965) method. The tubes containing the extracts were subjected to the procedures explained in the method (Lowry *et al*., 1951) and then, their absorbances were read against blind at 725 nm in the spectrometer, as described in the method. Standard curve prepared in advance were utilized to calculate the protein amount (mg g^{-1}. fresh weight). A modified version of the method of Heath and Packer (1968) was used to determine the amount of lipid peroxidation (MDA) leaves.

Statistical analysis: All experiments were conducted in triplicate. The results were subjected to statistical analysis by measuring the standard deviation of the mean and using analysis of variance (SPSS 15.0 for Windows Evaluation, one-way ANOVA- Duncan test).

RESULTS

As understood from the results obtained after the treatment of pepper seedlings with SA for 16 h, 1.5 mM SA application encouraged increase in seedling length, relative to control group seedlings (13.58%) ($p<0.05$) but did not affect leaf length ($p>0.05$). Five mM SA application was observed to delay the increase in seedling length, in comparison to control group seedlings (16.46%) ($p<0.05$) but it did not affect leaf length ($p>0.05$). Ten mM SA application was shown to delay seedling length increase (21.76%) and leaf length increase (36.66%) ($p>0.01$) (Table 1).

The 1.5 mM SA application was found to have a positive effect on fresh weight increase (12.40%) ($p<0.05$) but not on dry weight increase ($p>0.05$). 5 mM SA application was established to exercise a negative effect on fresh weight increase (14.28%) and dry weight increase (18.48%) ($p<0.05$). 10 mM SA application negatively influenced fresh weight increase (25.93%) and dry weight increase (34.12%) ($p<0.01$) (Table 2).

The 1.5 mM SA application was established to bring about an increase in chlorophyll (a+b) (15.31%) and protein (16.64%) content ($p<0.05$). 5 Mm SA application was also found to cause reduction in chlorophyll (a+b) (13.01%) and protein (24.64%) ($p<0.05$) content. Ten mM SA application was found to lead to significant reduction in chlorophyll (a+b) (23.33%) and protein (39.81%) ($p<0.01$) content (Table 3).

The 0.3 and 1.5 mM SA applications led to a decrease in the amount of MDA in comparison to the control but these data were found to be statistically insignificant ($p>0.05$). Similar to 5 and 10 mM SA applications led to a increase in the amount of MDA in comparison to the controls, these data were found statistically insignificant ($p>0.05$) (Table 3). Although 0.3 mM SA application also produced variations in all parameters, relative to the control group seedlings, the difference was not found to be statistically significant ($p>0.05$).

Table 1: Seedling and leaf lengths found in pepper (*Capsicum annuum* L.) seedlings treated with SA for 16 h

Concentrations (SA) (mM)	Seedling length (cm)	Leaf length (cm)
0	24.30±1.92	9.00±2.23
0.3	25.70±1.89	9.10±2.38
1.5	27.60±0.90*	9.40±1.19
5	20.30±1.20*	7.82±1.17
10	19.01±1.58**	5.70±0.44*

Significance level *: $p<0.05$, **: $p<0.01$

Table 2: Fresh and dry weights found in pepper (*Capsicum annuum* L.) seedlings treated with SA for 16 h

Concentrations (SA) (mM)	Fresh weight (g)	Dry weight (g)	DW mg/g FW
0	0.266±0.01	0.0211±0.002	79.32±1.88
0.3	0.295±0.01	0.0235±0.002	79.66±1.78
1.5	0.299±0.01*	0.0241±0.003	80.60±1.40
5	0.228±0.02*	0.0172±0.001*	75.43±1.30*
10	0.197±0.02**	0.0139±0.001**	70.02±1.65**

Significance level * :$p<0.05$, **: $p<0.01$

Table 3: Chlorophyll (a+b), protein and MDA amounts found in pepper (*Capsicum annuum* L.) seedlings treated with SA for 16 h

Concentrations (SA) (mM)	Chlorophyll (a+b) (mg g^{-1} fresh weight)	Protein (mg g^{-1} fresh weight)	MDA (nmol g^{-1} fresh weight)
0	2.083±0.16	1.826±0.19	12.77±3.87
0.3	2.278±0.14	2.026±0.17	10.71±2.19
1.5	2.402±0.13*	2.130±0.10*	11.33±2.37
5	1.812±0.10*	1.376±0.13*	16.18±2.56
10	1.597±0.16**	1.099±0.10**	14.73±2.04

Significance level *: $p<0.05$, **: $p<0.01$

DISCUSSION

No studies on the effect of SA on pepper seedlings could be traced from the literature available. It is known that plants exposed to stress show reduction in growth parameters, reduced protein synthesis or increased protein destruction and earlier aging due to critical metabolic changes like chlorosis (Vaadia *et al*., 1961). Earlier scientists also could observe stress-associated changes in the case of high SA concentrations (Çanakci and Munzuroglu, 2002). Low SA concentrations remained either ineffective or showed positive effects in terms of seedling growth (Khan *et al*., 2003). The inhibitory effect produced by high SA concentrations was found to be much more dominant than the stimulating effect of low SA concentrations. As we have already noted, lipid peroxidation is a central parameter that increases with environmental stress. The available data indicate that SA concentrations do not affect the amount of MDA (Gautam and Singh, 2009) on pepper seedlings.

The results brought about by high SA concentrations may be attributed to osmotic water correlations (Larque-Saavedra, 1978) intercellular space and stress due to a toxic effect. The decrease caused by high concentrations of SA in chlorophyll amount was claimed to have resulted from inhibition of chlorophyll biosynthesis, acceleration of chlorophyll destruction or both (Yang *et al*., 2002; Popova *et al*., 2009). It is known that SA and other salicylates have got an effect on the protein synthesis mechanism that could be described as a control mechanism (Fariduddin *et al*., 2003; Singh *et al*., 2010). It can be assumed that low SA concentrations inhibit ethylene biosynthesis (Huang *et al*., 1993; Srivastava and Dwivedi, 2000) while high SA concentrations stimulate it. The same mechanisms may be operating in opposite directions in a hormone-enzyme relation (Schneider and Whitman, 1974) depending on the SA concentration applied to the plant. Results showed that the toxic stress can be claimed for pepper seedlings (pigment and protein loss) but that oxidative stress is unclear (Gautam and Singh, 2009; Krantev *et al*., 2008; Popova *et al*., 2009).

CONCLUSION

Salicylic acid is an effective precursor molecule that builds resistance against abiotic and biotic stress in plants. It produces a similar effect depending on the concentration of the exogenously applied SA and the type of the plant. However when applied individually and at high concentrations, SA may be a toxic stress factor for the plant. In conclusion, these physiological effects of SA which is regarded a new growth regulator, should be considered at the hormonal dimension.

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