Genetics & Applications

Institute for Genetic Engineering and Biotechnology University of Sarajevo

Research article Open access

The Effect of Salicylic Acid on Response to Water Deficit in Basil

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DOI: 10.31383/ga.vol2iss1pp42-48

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Received
February, 2018
Accepted
May, 2018
Published
June, 2018

Abstract

Drought-induced stress is the main limiting factor that affects growth and development in plants. In this study, the effect of exogenous application of salicylic acid (SA) on drought tolerance in basil was investigated. The analysis showed that application of SA under drought stress had significant effects on physiological and biochemical parameters, such as photosynthetic pigments content, total phenolics, flavonoids, flavanols and protein content and peroxidise activity, but had no significant effects on the morphological parameters, such as stem length, length and areaof leaves. In drought conditions, total phenolics and peroxidase activity reduced significantly, but all photosynthetic pigments, total flavonoids, flavanols and proteins increased significantly. Application of SA displayed some alleviating effects against drought induced stress through increase of plant growth, total flavonoids content and peroxidase activity.

Keywords

drought, salicylic acid, morphological and physiological parameteres

Introduction

Drought is one of the most important abiotic stresses, and drought tolerance is a complex property of some species which is determined by different morphological and/or physiological characteristics. Water stress involves loss of water, leading to stomatal closure and limited gas exchange (Amarjit et al., 2005; Shao et al., 2008). The first sign of water deficit is the reduction of turgor, which leads to disorders in growth processes. However, the consequences of stress are not manifested at the morphological level only, but also on physiological (growth inhibition, stomatal closure, decreased transpiration rate, water potential and photosynthesis rate) as well as biochemical and molecular levels (accumulation of organic compounds and abscisic acid, endogenous phytohormone and lipids, changes in expression of stress-responsive genes, etc.) (Pál et al., 2013). Plants can respond and adapt to water stress by altering their cellular metabolism to

activate various defense mechanisms including accumulation of secondary metabolites and synthesis of osmoprotectants, such as proline and soluble sugars (Fayez & Bazaid, 2014). Additionally, proline is usually accumulated in plants (more than other amino acids) under drought stress (Irigoyen et al., 1992) while soluble sugars accumulate in salt and drought stressed plants (Pinheiro et al., 2004; Munns, 2005).

Salicylic acid (SA) is phenolic phytohormone, which plays a regulatory role in numerous plant physiological processes such as growth and development (Vicente & Plasencia, 2011; Jakhar & Sheokand, 2015), photosynthesis (Hayat et al., 2012; Jakhar & Sheokand, 2015), absorption and transport of the ions, seed germination, glycolysis (Rajjou et al., 2006; Vicente & Plasencia, 2011). Several works support the role of SA in response to drought (Singh & Usha, 2003; Mardani et al., 2012; Fayez & Bazaid, 2014; Khan et al., 2015). Exogenously applied SA can regulate enzymatic and non-enzymatic defense system in order to increase plant tolerance to abiotic stress (He et al., 2002; Erasalan et al., 2007; Alam et al., 2013).

The presented study was conducted in order to examine whether exogenous application of SA could improve drought tolerance in basil plants. Plant mass, leaf area, content of photosynthetic pigments, proline, secondary metabolites, soluble sugars and total protein as well as and peroxidase activity were examined.

Materials and methods

Plant materials and treatments

Seeds of basil (*Ocimum basilicum* L.) were obtained commercially (Green Paradise S.R.L) and sown in pots containing air dried soil. The pots were watered every day up to four weeks after sowing. The plants with two developed leaves similar in size were selected and transplanted into plastic containers measuring 10 cm in width and containing about 3 kg of air dried soil. Plants were divided into four groups as follows: 1) Plants that were watered daily (control plants), 2) plants sprayed with 1 mM SA + watered daily, 3) plants sprayed with 1 mM SA + water deprivation and 4) plants deprived of water without SA treatment. SA was sprayed once in one concentration (1 mM) until both sides of leaves were wet. Ten days after salicylic acid application plant material was harvested (six plants from each replication) for further analysis.

Morphological analysis and plant mass

All plants were carefully removed from soil and roots were washed to remove soil residues. Stem and leaf length, and leaf area were measured and calculated respectively using ImageJ 1.43v (Wayne Rasband, National Institutes of Health, USA; http://rsb.info.nih.gov/ij) software. Water content and dry weight (dried at 65°C for 72 hours) was recorded.

Photosynthetic pigments content

Photosynthetic pigments were analysed from 80% acetone extract by absorbance reading at 663, 646 and 440 nm according to the Arnon (1949) and quantified according to Porra et al. (1989) and Holm (1954):

Chlorophyll *a* = $12,25*A_{663}–2,55*A_{646}$ (μg/mL)
Chlorophyll *b* = $20,31*A_{646}–4,91*A^{663}$ (μg/mL)
Total Chlorophylls = $17,76*A_{646}–7,34*A_{663}$ (μg/mL)
Carotenoids = $4,69*A_{440}–0,267*(A_{663}*A_{646})$ (μg/mL)
Pigment content was expresed as mg/gDW

Proline content

Proline content was determined according to Carillo et al. (2008). Dried leaf material (50 mg) was homogenized in 5 mL of ethanol:water mixture (60:40) and homogenate was incubated for 24h at +4°C. After centrifugation at 10 000 rpm supernatant was collected. 500 μL of supernatant was mixed with 1000 mL of 1% acid ninhydrin. The reaction mixture was placed in water bath at 95°C for 20 minutes, cooled to room temperature and the absorbance was measured at 520 nm using proline as a standard. Proline content was expressed as mg proline/g DW.

Secondary metabolite analysis

Phenolics were extracted from dried plant material, by maceration in 80% methanol (HPLC grade) and incubation for 24h at +4° C. Extracts were centrifuged at 2000 rpm for 15 min, and supernatants were collected for further analysis. Total phenolic content was analysed according to Wolfe et al. (2003) using Folin-Ciocalteu reagent and gallic acid as a standard. The results were expressed as mg of gallic acid equivalent per g of

dry weight (mgGAE/gDW). Total flavonoid content was determined using aluminium chloride method (Ordonez et al., 2006) and catechin as standard. The results were expressed as mg of catechin equivalent per g of dry weight (mgCE/gDW). Total flavanol content was analysed according to modified method of Gadzovska et al. (2007) using 1% DMACA reagent (p-dimethyl aminocinnamaldehyde in HCl:CH3OH, 8:92) and catechin as standard. The results were expressed as mg of catechin equivalent per g of dry weight (mgCE/gDW).

Soluble sugar content

Dry samples (0.1 g) were macerated and extracted with 2.5 ml of 80% ethanol at 90°C for 60 min, followed by centrifugation at 10 000 rpm at 4°C for 10 minutes. The process was repeated for complete extraction. Total soluble sugar content was determined using anthrone reagent and glucose as standard (Roe, 1955). Results were expressed as mg soluble sugar/g DW.

Enzyme activity and total protein content

The fresh plant material was homogenized with 5mL of 50 mM sodium phosphate buffer (pH 7). The homogenate was centrifuged at 10 000 rpm for 15 min at 4°C. The supernatant was used for enzyme assays.

The total protein content was determined according to Bradford (1976) with spectrophotometric absorbance reading at 595 nm, using BSA (Bovine Serum Albumin) as a standard. The protein content was expressed as mg of protein per g of fresh weight.

Peroxidase activity (POD) was determined based on an increase in absorbance at 420 nm as described by Gonzales et al. (1984). The results were recorded as μcat peroxidase activity.

Statistical analysis

All obtained data were analysed for significant differences using factorial analysis of variance. Statistical analysis was performed using Statistica 10 software and the means were compared using Newman-Keuls Test at $p<0,05$.

Results and Discussion

Application of salicylic acid when plants were exposed to drought increased stem and leaf length as well as the leaf area of basil (Table 1) but these differences were not statistically significant. Negative effect of water deficiency on morphological parameters of basil can be alleviated by applying salicylic acid as previouslly recorded (Hussain et al., 2008; Farooq et al.,2009; Habibi, 2012; Kang et al., 2013; Kordi et al. 2013; Fayez & Bazaid, 2014).

The statistical analysis showed that SA application induced significant changes in photosynthetic pigments under drought (Table 2). Increase of chlorophylls as a result of drought stress was diminished when SA was applied. Drought stress induced increase of chlorophyll a, b, total chlorophylls and carotenoides comparing with control plants. Chlorophylls, esspecially chlorophyll a content, increased also in plants treated with SA not exposed to drought stress. Similar results were obtained by Bagherifard et al. (2015), where salinity in combination with SA reduced chlorophyll level. Pigment content decline was reported in drought stressed plants (Massacci et al., 2008), which was associated with decline in Rubisco activity and reduced gas exchange (Bota et al., 2004) and instability of protein complexes and destruction of chlorophyll by increased activity of chlorophyll degrading enzymes under stress (Kordi et al., 2013). Noumerous publications report that SA has a protectives role in photosynthesis and enhances Rubisco activity in water stressed plants (Idrees et al., 2010). However, the reverse effects of SA are also known, where SA serves as a stressor that can negatively affect the photosynthetic process. These effects are usually asociated with different used concentrations but cases are recorded where the same applied concentration exerted different effects in different plant species or in different conditions of the environment (Pancheva et al., 1996; Janda et al., 1999; Ananieva et al., 2002).

The proline and carbohydrate content was not significantly affected by SA treatment in plants under drought stress (Table 3). Statistically significant variation was noticed in secondary metabolite production. The results showed that the highest phenol content (2,672 mg/gDW) was in control plants and plants sprayed with SA that were exposed to drought (2,648 mg/gDW). Plants exposed to drought showed increase in flavonoid content by 73,53, while for plants exposed to drought and sprayed with SA flavonoid content was

Table 1. Effect of salicylic acid and drought stress on stems length, the surface and the length of the leaves, fresh and dry weight of basil

Treatment	Stem length (mm)	Leaves length (mm)	Leaf area (mm^2)	Water content %	Dry weight (g)
Drought+SA	29,609[a] (±2,373)	11,929[a] (±1,302)	90,375[a] (±17,603)	90.06 (±0,01)	0.098 (±0,025)
Drought without SA	27,267[a] (±5,313)	8,430[a] (±3,370)	47,317[a] (±41,476)	82.77 (±0,01)	0.055 (±0,011)
Watered+SA	23,934[a] (±3,409)	12,033[a] (±1,329)	97,586[a] (±25,973)	91.98 (±0,01)	0.103 (±0,036)
C	22,091[a] (±2,834)	9,959[a] (±0,711)	70,984[a] (±9,778)	91.08 (±0,01)	0.067 (±0,011)

Drought + SA - drought stress with application of 1mM SA; Drought without SA - drought stress without application of SA ; Watered+SA - application of 1 mM SA+ watering every day; C- control (Plants were watered every day);
Data are the mean of three independent replicates (± standard error).
Means within a parameter not sharing the same letter differ significantly at p<0,05

Table 2. Effect of salicylic acid and drought stress on chlorophyll *a* and chlorophyll *b*, total chlorophyll and carotenoids content of basil

Treatment	Chlorophyll *a* mg/g DW	Chlorophyll *b* mg/g DW	Total chlorophylls mg/g DW	Carotenoids mg/g DW
Drought+SA	4,6114[d] (±0,021)	2,1963[d] (±0,010)	6,8781[d] (±0,031)	0,9251[d] (±0,004)
Drought without SA	6,6823[a] (±0,006)	3,1681[a] (±0,019)	9,9525[a] (±0,025)	1,4743[a] (±0,001)
Watered+SA	5,8022[b] (±0,021)	2,2963[c] (±0,009)	8,1866[b] (±0,030)	1,1814[b] (±0,003)
C	5,5227[c] (±0,030)	2,4020[b] (±0,021)	8,0088[c] (±0,052)	1,0857[c] (±0,000)

Drought + SA - drought stress with application of 1mM SA; Drought without SA - drought stress without application of SA ; Watered+SA - application of 1 mM SA+ watering every day; C- control (Plants were watered every day);
Data are the mean of three independent replicates (± standard error).
Means within a parameter not sharing the same letter differ significantly at p<0,05

68,47% Total flavanols content was significantly increased only under drought. It has already been noted that stress leads to an increase in polyphenols (Ksouri et al., 2007; Bagherifard et al., 2015). Phenolic compounds in plants are generally affected by environmental stresses (Kim et al., 2006; Giorgi et al., 2009). The content of secondary compounds in plants can be dependent on the environmental conditions and have strong impact on the metabolic pathways responsible for the accumulation of the related natural products (Ramakrishna & Ravishankar, 2011).

Significant increase in protein content was noticed only under drought stress. Peroxidase activity was significantly higher when plants were sprayed with SA, while exposure of plants to drought, irelevant to SA application, induced decrease of peroxidase activity (Table 3). Increase of peroxidase activities in plants sprayed with SA are probably result of activation of defense mechanisms after SA treatment as noted before (Karalija & Parić, 2017). It has been previously noted that SA promotes enzymes involved in the plant's defense system and its exogenous application, and its role in plant-defence system under stress conditions has been studied in many plants (Hayat et al., 2008; Zhao et al., 2009; Idrees et al., 2010; War et al., 2011; Khan et al., 2015).

Conclusions

The presented study showed that application of SA can have beneficiarry effects on basil plants when exposed to drought such as increase of growth. Optimisation of best SA concentration is necessary in order to get the best results. Increase in secondary metabolite content showes

Table 3. Effect of salicylic acid and drought stress on proline, secondary metabolites, soluble sugar, total protein content and peroxidise activity

Treatment	Proline mg/gDW	Total Carbohydrates mg/gDW	Total Phenols mg/gDW	Total Flavonoids mg/gDW	Total Flavanols mg/gDW	Total Proteins mg/gDW	POX SA60	POX SA120
Drought+SA	0,073[a] (±0,013)	392,3304[a] (±46,037)	2,648[a] (±0,299)	12,283[a] (±0,163)	0,450[b] (±0,037)	0,582[b] (±0,116)	0,232[bc] (±0,025)	0,126[bc] (±0,006)
Drought without SA	0,088[a] (±0,006)	443,1768[a] (±5,527)	1,815[b] (±0,058)	14,633[a] (±2,247)	0,651[a] (±0,048)	1,199[a] (±0,045)	0,197[c] (±0,015)	0,102[c] (±0,018)
Watered+SA	0,067[a] (±0,003)	437,2104[a] (±82,284)	0,722[c] (±0,102)	4,494[b] (±0,089)	0,444[b] (±0,022)	0,476[b] (±0,091)	0,403[a] (±0,013)	0,221[a] (±0,007)
C	0,069[a] (±0,006)	416,5128[a] (±20,377)	2,672[ab] (±0,425)	3,873[b] (±0,562)	0,366[b] (±0,010)	0,507[b] (±0,079)	0,279[b] (±0,013)	0,149[b] (±0,015)

Drought + SA - drought stress with application of 1mM SA; Drought without SA - drought stress without application of SA; Watered+SA - application of 1 mM SA+ watering every day; C- control (Plants were watered every day);
Data are the mean of three independent replicates (± standard error).
Means within a parameter not sharing the same letter differ significantly at p<0,05

activation of non-enzymatic antioxidant plant systems as a response to drought induced oxidative stress.

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