Ecotoxicology and Environmental Safety 106 (2014) 232–238

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

The physiological and biochemical effects of salicylic acid on sunflowers (*Helianthus annuus*) exposed to flurochloridone

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ARTICLE INFO

Article history:
Received 13 August 2013
Received in revised form
28 April 2014
Accepted 29 April 2014
Available online 21 May 2014

Keywords:
Flurochloridone
Salicylic acid
Helianthus annuus
Antioxidant enzymes
Malondialdehyde
Pigments

ABSTRACT

In this study, we comparatively evaluated the effects of the flurochloridone as well as flurochloridone and exogenously applied salicylic acid (SA) on *Helianthus annuus* L. to find out herbicide-induced toxicity reducing influence of SA. We examined and compared the physiological and biochemical effects of different concentrations of flurochloridone (11, 32 and 72 mM) in both the SA pre-treated and non-treated plants. The plants treated with flurochloridone exhibited reduced total chlorophyll, carotenoid, and relative water content compared to the control group, whereas the plants that were pre-treated with SA exhibited relatively higher values for the same physiological parameters. In the SA non-treated plants, the superoxide dismutase, glutathione reductase and glutathione S-transferase activities were increased in the treatment groups compared to the control group. In the treatment groups, these enzyme activities were decreased in the SA-pre-treated plants compared to the non-treated plants. Ascorbate peroxidase and catalase activities decreased in the flurochloridone-treated plants compared to the control plants. The ascorbate peroxidase activity increased in the control groups but decreased in the treatment groups in the SA pre-treated plants compared to the non-treated plants. However, SA treatment decreased the activity of catalase in the control and treatment groups compared to the plants that were not treated with SA. Flurochloridone treatment increased the malondialdehyde content in the treated groups compared to the control groups, whereas SA-pretreatment decreased malondialdehyde content compared to plants that were not treated with SA. Flurochloridone treatment increased endogenous SA content compared to the control. Although the residual levels of herbicide in the plants increased proportionately with increasing herbicide concentrations, the SA-pre-treated plants exhibited reduced residual herbicide levels compared to the plants that were not treated with SA. These results indicate that the flurochloridone induces various physiological and biochemical responses in non-target plants and that treatment with exogenous SA can increase stress resistance by altering these responses.

1. Introduction

Herbicides are xenobiotic substances that control the development and the growth of weeds. Apart from being structurally diverse, all herbicides can induce harmful effects upon contact with plants (Lukatkin et al., 2013). Various physiological and biochemical responses, including chlorosis, lipid peroxidation (LPO) and antioxidant responses, can occur in plants as a consequence of herbicide exposure (Doganlar, 2012). Lipid peroxidation affects the physiological process of the cell (Labudda, 2013). Malondialdehyde (MDA), which is a metabolic product of lipid peroxidation, is a widely accepted indicator of oxidative damage in plants (Smirnoff, 1993). Herbicide tolerance in plants is attributed to the antioxidant system (Alla and Hassan, 2006). The antioxidant system functions to scavenge the toxic radicals that are produced during oxidative stress and to facilitate plant survival during stress conditions. The antioxidative system comprises both enzymatic and non-enzymatic systems. The non-enzymatic system includes ascorbic acid (vitamin C), α-tocopherol, glutathione and carotenoids, whereas the enzymatic system includes superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione S-transferase (GST), etc. (Mandal et al., 2009). SOD functions by scavenging the

Abbreviations: APX, ascorbate peroxidase; AsA, ascorbate; CAT, catalase; GSH, glutathione; GST, glutathione S-transferase; GR, glutathione reductase; LPO, lipid peroxidation; MDA, malondialdehyde; RWC, relative water content; SA, salicylic acid; SOD, superoxide dismutase

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http://dx.doi.org/10.1016/j.ecoenv.2014.04.041
0147-6513/

superoxide radical, whereas CAT and APX function by scavenging hydrogen peroxide (H_2O_2) (Bowler et al., 1992). GR efficiently maintains the reduced pool of glutathione (Gill et al., 2013). In plants, GST catalyzes the conjugation of glutathione to xenobiotic substances. Thus, the antioxidant system plays a crucial role in the herbicide detoxification (Mitsou et al., 2006). In plants, carotenoids play an important photoprotective role by scavenging reactive oxygen species (ROS) and by suppressing LPO (Gill and Tuteja, 2010). In addition, chlorophylls are the fundamental pigments that play a role in photosynthesis, and differences in plant leaf chlorophyll content reflect their resistance levels and photosynthetic capacities (Carter and Spiering, 2002; Golkar et al., 2009).

Antioxidant capacity can be insufficient to prevent the harmful effects of stress when the intensity of the stress increases. Thus, the production of signaling molecules, such as ethylene, jasmonic acid (JA) and SA, is important (Kadioglu et al., 2011). SA, which is a plant phenolic compound, is an important signal molecule that plays a role in regulating the plant responses given to environmental stresses (Baghizadeh and Hajmohammadrezaei, 2011). SA might affect plant antioxidant responses by inhibiting or activating antioxidant enzymes. For this reason, SA can modulate plant responses against numerous abiotic stress factors such as drought, cold, heat and herbicide-induced stress (Larkindale and Knight, 2002; Scott et al., 2004; Kadioglu et al., 2011; Radwan, 2012).

Flurochloridone is a selective herbicide that is commonly used to inhibit weeds that occur during the growth of the crop plants, such as grains, sunflowers and potatoes. Flurochloridone, which is absorbed by the roots and stems, can affect the biosynthesis of carotenoids, chlorophyll and abscisic acid metabolites (Lay et al., 1985; Kličova et al., 2002; Nikoloff et al., 2012).

The aim of this study is to determine physiological and biochemical changes resulting from implementation of flurochloridone on sunflower plants. We also investigated the effects that are elicited on these parameters by the treatment of exogenous SA. In this study, we tried to answer if the toxic effects elicited on sunflower plants by flurochloridone treatment can be ameliorated by pre-treatment with SA or not.

2. Material and methods

2.1. Plant growth and treatments

In this study, the flurochloridone herbicide was provided by "Safa Agriculture", and the sunflower seeds were provided by "May Seed Growing." The seeds were planted after a portion of the plants was incubated for six hours in distillated water, whereas another portion was incubated for six hours in a 0.5 mM SA solution. The plants were grown in pots containing perlite, in triplicate, using Hoagland solution (Hoagland and Arnon, 1938). On the 21st day of the growth, flurochloridone was sprayed on both groups of plants, one of which was SA-treated while the other was SA-untreated. The doses used were determined from the toxicity testing (11, 32 and 72 mM). Some leaves, gathered on the fifteenth day after the treatment, were stored at −80 °C until further various physiological and biochemical parameters were investigated, whereas some fresh leaves were used for evaluating the relative water content (RWC).

2.2. Determination of total chlorophyll and carotenoids levels

We extracted pigment contents as previously described by De-Kok and Graham (1980). 1 g of leaf tissue was homogenized using a glass mortar with 50 mL acetone (100 percent, Merck) and then centrifuged. Absorbance values of the centrifuged samples were measured as described previously by Lichtenthaler and Welburn (1983) at 662, 645 and 470 nm.

2.3. Relative water content (RWC) determination

The fresh weights of the leaves were determined. Next, the leaves were rendered turgid by incubation in water for four hours. After measuring the turgid weights of the leaves, their dry weights were measured after being dried at 70 °C for 48 h in incubator. The RWC percent values were calculated using the formula below (Barr and Weatherley, 1962).

$$[(F.W.–D.W.)/(T.W.–D.W.)] \times 100$$

2.4. Extraction of plant leaves

0.5 g of leaf tissue was homogenized in 2.5 mL 0.1 M pH 7.5 Tris–HCl tampon, 2.5 mL 0.1 mM EDTA and 0.5 mL 1 percent PVP. The homogenates were centrifuged at 4 °C and 18,000 rpm and for 30 min. The supernatants were stored in a −80 °C deep freezer (Andrews, 2005). The activities of all of enzymes were expressed as specific activities.

2.5. Determination of CAT activity

We spectrophotometrically measured the activity of CAT, as previously described by Luck (1963), by measuring the decrease of absorbance at 240 nm attributed to the decomposition of H_2O_2. The reaction mixture consisted of 800 μL of 1/15 M phosphate buffer (pH 7) containing H_2O_2, and 200 μL of extracted leaf tissue.

2.6. Determination of SOD activity

We measured the activity of SOD, as previously described by McCord and Fridovich (1969), in terms of the ability of the enzyme to prevent the reduction of cytochrome c by superoxide generated by the xanthine oxidase system. The SOD activity was calculated from the calibration curve of bovine erythrocyte SOD. The enzyme activity was determined using a spectrophotometer (Biochrom Libra S22) at 550 nm.

2.7. Determination of GST activity

We spectrophotometrically measured the activity of GST, as previously described by Habig et al. (1974). The final reaction mixture consisted of 400 μL of 0.1 M phosphate buffer, 400 μL of 0.002 M GSH, 100 μL of extract and 150 μL of 0.15 M CDNB. The reactions were monitored using a spectrophotometer (Biochrom Libra S22) at 344 nm.

2.8. Determination of GR activity

We determined the activity of GR by the method described by Carlberg and Mannervik (1985). The reaction mixture consisted of 500 μL of 0.2 mM potassium phosphate buffer (pH 7), 50 μL of 2 mM NaDPH, 50 μL of 20 mM GSSG, 100 μL of distillated water and 300 μL of extract. The reactions were monitored using a spectrophotometer (Biochrom Libra S22) at 340 nm.

2.9. Determination of APX activity

We measured the activity of ascorbate peroxidase as previously described by Nakano and Asada (1981) and Cakmak (1994). 0.5 g of leaf tissue was homogenized in 10 mL of 50 mM potassium phosphate buffer (pH 7.6). Next, the homogenate was centrifuged at 20,000 rpm for 15 min. The reaction mixture contained 550 μL of phosphate buffer (pH 7.6), a mixture of 12 mM of H_2O_2 and 100 μL of 10 mM EDTA, 100 μL of the extract and 250 μL of 0.25 mM ascorbic acid. The reaction was monitored using a spectrophotometer (Biochrom Libra S22) at 290 nm.

2.10. Determination of total soluble protein

We determined the total soluble protein content as previously described by Bradford (1976) using BSA as a standard. We spectrophotometrically measured the reactions (Biochrom Libra S22) at 595 nm.

2.11. Determination of MDA content

We measured MDA content as previously described by Heath and Packer (1968). Leaf tissue (0.5 g) was homogenized in 5 mL 0.1 percent mL trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 5 min. Next, 2 mL of this solution and 2 mL of 0.5 percent thiobarbituric acid (TBA) were boiled in a 95 °C boiling water bath for 30 min (TBA was prepared in twenty percent TCA). After boiling, the samples were cooled in an ice-bath. The final mixture was centrifuged at 10,000 rpm for 15 min. We measured the absorbances of the supernatants at 532 and 600 nm. The measurements made at 600 nm were deduced from those at 532 nm, and the levels of MDA were calculated with a 155 mM^{-1} cm^{-1} extinction coefficient.