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Alleviation of isoproturon toxicity to wheat by exogenous application of glutathione

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ABSTRACT

Treatment with the recommended field dose of isoproturon to 7-d-old wheat seedlings significantly decreased shoot height, fresh and dry weights during the subsequent 15 days. Meanwhile contents of carotenoids, chlorophylls and anthocyanin as well as activities of δ-aminolevulinate dehydratase (ALA-D), phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) were significantly inhibited. On the other hand, the herbicide significantly increased malondialdehyde (MDA), a naturally occurring product of lipid peroxidation and H_2O_2, while it significantly decreased the contents of glutathione (GSH) and ascorbic acid (AsA) and reduced the activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). These findings indicate an induction of a stress status in wheat seedlings following isoproturon treatment. However, exogenous GSH appeared to limit the toxic effects of isoproturon and seemed to overcome this stress status. Most likely, contents of pigment and activities of enzymes were raised to approximate control levels. Moreover, antioxidants were elevated and the oxidative stress indices seemed to be alleviated by GSH application. These results indicate that exogenous GSH enhances enzymatic and nonenzymatic antioxidants to alleviate the effects of isoproturon.

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1. Introduction

Isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea] is a urea-type, photosynthetic inhibiting herbicide. The herbicide blocks the forward electron flow. Charge recombination can lead to the formation of triplet chlorophyll which can then react with O_2 leading to the formation of $^1O_2$ [1–5]. Reactive oxygen species (ROS) typically result from the excitation of O_2 to form $^1O_2$ or from the transfer of one, two or three electrons to O_2 to form, respectively, a superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) or a hydroxyl radical (HO^-). They react with lipids, proteins, pigments and nucleic acids and causing lipid peroxidation, membrane damage and inactivation of enzymes [6–8]. Isoproturon inhibited growth and induced oxidative stress in wheat [8] and maize [9,10]. However, plants have a protective system for ROS scavenging composed of non-enzymatic antioxidants such as glutathione (GSH) and ascorbate (AsA) and enzymatic antioxidants including superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) [6,10,11]. Plant tolerance to some herbicides is regulated through pivotal participation of glutathione [12]. CAT and SOD activities increased in response to xenobiotics [13]. Moreover, chlorophyll biosynthesis could be influenced by the photosynthetic inhibitors. In chlorophyll biosynthesis, two molecules of δ-aminolevulinate are combined by δ-aminolevulinate dehydratase (ALA-D, EC 4.2.1.24) to form porphobilinogen. In addition, secondary metabolism is also affected by herbicides. Phenylalanine ammonia lyase (PAL, EC 4.3.1.1) and tyrosine ammonia lyase (TAL, EC 4.3.1) are considered as switches for the the production of secondary metabolites as terpenoids, isoflavonoids, anthocyanin, etc. [14]. So, the influence of herbicides on plant tolerance could result from changes in primary and secondary metabolism that might be due to alterations in antioxidant system. Therefore, the present work was aimed to evaluate the exogenous supplementation of GSH, as an antioxidant in order to ascertain the alleviation of isoproturon toxicity to wheat.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of wheat (*Triticum aestivum*, Giza 157) were surface sterilized in 3% sodium hypochlorite solution for ten min, thoroughly washed, soaked for 8 h and germinated in sand/clay soil (1:1, v/v) in plastic pots (25 cm diameter × 20 cm height). The pots were kept at 12 h photoperiod with 400–450 μmol m^{-2} s^{-1}

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photosynthetic photon flux density, 75–80% relative humidity, and 22/10 °C day/night regime. After 7 days, the seedlings were thinned to only four per pot and one-fourth strength Hoagland solution was used for irrigation. Pots were divided into three groups; two for isoproturon treatment at the recommended field dose (2.5 l ha^{-1}) either alone or with GSH and the third was left to serve as control. GSH was applied only once and carried out by supplementing 50 ml of Hoagland solution with 5 mM GSH. The quantity of the herbicide and/or GSH (suitable for pot surface area) was mixed in an amount of water enough to spray the surface area of seedlings without excluding soil in pots twice, in one direction and cross-wise. Shoots were collected at this stage (zero time) and after 5, 10 and 15 days from treatment, rinsed with copious amounts of tap water, dried by blotting with paper towels and fresh weights as well as shoot height were measured, and then dried at 70 °C for dry matter measurements.

2.2. Determination of photosynthetic pigments and anthocyanin

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were extracted in 85% acetone and determined spectrophotometrically [15]. Anthocyanin was extracted in acidic methanol (HCl, 1% v/v) and the absorbance was read at 525 nm and 585 nm [16].

2.3. Determination of lipid peroxides and H_2O_2

Lipid peroxides (as malondialdehyde, MDA) were extracted in 150 mM KCl and centrifuged at 7000*g* for 15 min. One ml of the supernatant was incubated at 37 °C for 2 h with 1 ml of 0.6 M trichloroacetic acid (TCA), mixed and centrifuged at 5000*g* for 10 min. One ml of supernatant was taken with 1 ml of thiobarbituric acid and placed in a boiling water bath for 10 min, cooled and diluted with 1 ml distilled water and the absorbance was read at 535 nm [17]. H_2O_2 was extracted in 200 mM perchloric acid, centrifuged at 5000*g* for 10 min and the supernatant was neutralized with 4 M KOH. After centrifugation at 3000*g* for 5 min, 0.2 ml of the supernatant was loaded on 1 ml column of Dowex 1X8-100 anion exchange resin and eluted with 0.8 ml of distilled water. The assay mixture contained 0.4 ml 12.5 mM 3-dimethylaminobenzoic acid in 375 mM phosphate buffer pH 6.5, 0.08 ml 1.3 mM 3-methyl-2-benzothiazolinone hydrazone and 0.02 ml (0.25 units) horseradish peroxidase. The reaction was initiated by the addition of 1 ml extract and the increase in absorbance at 590 nm was monitored for 3 min [18].

2.4. Determination of glutathione and ascorbate (GSH and AsA)

GSH was extracted in TCA (5%, w/v) and 10 mM EDTA and centrifuged at 12,000*g* for 15 min. GSH was assayed in 100 mM phosphate buffer, pH 6.8 containing 10 mM EDTA, 1 mM 1-chloro-2,4-dinitrobenzene and 1.0 U equine glutathione-S-transferase and incubated at 35 °C for 30 min. The absorbance at 340 nm was recorded before commencing the reaction and after the reaction had run to completion [19]. AsA was extracted in 62.5 mM phosphoric acid and centrifuged at 12,000*g* for 20 min and filtered through a 0.5 μm Millipore filter. The filtrate was loaded onto an ion exclusion column (300 × 7.8 mm) connected to analytical HPLC system, and eluted with 4.5 mM H_2SO_4 at a flow rate of 0.5 ml min^{-1}. The elution of AsA was detected at 245 nm [20].

2.5. Enzyme activity assays

δ-Aminolevulinate dehydratase (ALA-D, EC 4.2.1.24) was extracted in chilled acetone, centrifuged and the residue was dried at room temperature. An aliquot of the residue was extracted with Tris–HCl buffer (0.05 M, pH 9) and centrifuged at 24,000*g* for 15 min. The reaction mixture contained 1.8 ml of the supernatant and 0.2 ml of a solution containing δ- aminolevulinate (5 mg ml^{-1}) in Tris–HCl buffer (0.05 M, pH 7.0). After incubation at 37 °C for an hour, the reaction was stopped by 1.0 ml of 0.1 M mercuric chloride in 10% trichloroacetic acid, an equal volume of modified Ehrlich's reagent was added and the absorbance was read at 555 nm [21]. The extraction and assay of phenylalanine ammonia lyase (PAL, EC 4.3.1.1) and tyrosine ammonia lyase (TAL, EC 4.3.1) were carried out according to Beaudoin-Egan and Thorpe [22]. Superoxide dismutase (SOD; EC 1.15.1.1) was extracted in 50 mM phosphate, pH 7.8, 0.1% (w/v) bovine serum albumin, 5.5 mM ascorbate, and 8 mM β-mercaptoethanol. SOD was assayed in 50 mM phosphate, pH 7.8, 9.9 mM L-methionine, 0.057 mM nitroblue tetrazolium (NBT), 0.025% (w/v) Triton X-100, and 0.1 mM riboflavin at 560 nm [23]. Catalase (CAT; EC 1.11.1.6) was extracted in 50 mM phosphate buffer, pH 7, and 1 mM dithiothreitol. CAT was assayed by determining the consumption of H_2O_2 at 240 nm in 50 mM phosphate buffer, pH 7.5 and 200 mM H_2O_2 [24]. Ascorbate peroxidase (APX; EC 1.11.1.7) was extracted in 0.1 M Tricine-KOH buffer, pH 8, 1 mM dithiothreitol, 10 mM $MgCl_2$, 50 mM KCl, 1 mM EDTA, 0.1% (w/v) Triton X-100, and 0.28 mM phenylmethylsulfonyl flouride. APX was evaluated at 270 nm in 50 mM phosphate buffer, pH 7.5, 40 mM Na ascorbate and 200 mM H_2O_2 [25].

Each experiment was repeated twice in triplicates, so that the mean was obtained from six replicates. The full data were statistically analyzed using ANOVA and LSD at $P < 0.05$.

3. Results

3.1. Growth parameters

Treatment with the recommended field dose of isoproturon to 7-d-old wheat seedlings significantly decreased shoot height below control values during the subsequent 15 days. The magnitude of decrease progressively augmented over time (Fig. 1). Meanwhile, shoot fresh weights and dry weights were significantly reduced by the herbicide during the whole experimental period with respect to the controls. However, exogenous GSH seemed to restrict reductions in growth parameters, diminishing the effects of isoproturon. Indeed, isoproturon-induced reductions of 15, 20 and 15% in shoot height, fresh and dry weight respectively were restricted to 5, 9 and 5%, correspondingly to exogenous application of GSH.

3.2. Pigments content and activity of δ-aminolevulinate dehydratase (ALA-D)

As well, the herbicide resulted in significant decreases in carotenoids, chlorophyll a and chlorophyll b of wheat seedlings during the whole experimental period as compared to the untreated controls (Fig. 2). The same was also detected for ALA-D activity. Carotenoids and ALA-D activity were more inhibited by the herbicide relative to chlorophyll a and b. Carotenoids, chlorophyll a, chlorophyll b and ALA-D activity were reduced by isoproturon on the 15th day by about 40, 27, 24 and 51%, respectively. Nonetheless, the presence of GSH generally retracted these reductions. It rendered the effects of the herbicide on carotenoids, chlorophyll a and chlorophyll b to become mostly insignificant during the whole experiment and on ALA-D activity only during the first 5 days of treatment. Despite these retractions, their magnitudes were not so great to nullify the herbicide effect on the 15th day. The presence of GSH lowered the magnitude of reduction in carotenoids, chlorophyll a, chlorophyll b and ALA-D activity on the 15th day to become only 14, 14, 15 and 23%, respectively.

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