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Research article

Effect of azoxystrobin fungicide on the physiological and biochemical indices and ginsenoside contents of ginseng leaves

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ABSTRACT

Background: The impact of fungicide azoxystrobin, applied as foliar spray, on the physiological and biochemical indices and ginsenoside contents of ginseng was studied in ginseng (*Panax ginseng* Mey. cv. "Ermaya") under natural environmental conditions. Different concentrations of 25% azoxystrobin SC (150 g a.i./ha and 225 g a.i./ha) on ginseng plants were sprayed three times, and the changes in physiological and biochemical indices and ginsenoside contents of ginseng leaves were tested.

Methods: Physiological and biochemical indices were measured using a spectrophotometer (Shimadzu UV-2450). Every index was determined three times per replication. Extracts of ginsenosides were analyzed by HPLC (Shimadzu LC20-AB) utilizing a GL-Wondasil C_{18} column.

Results: Chlorophyll and soluble protein contents were significantly ($p = 0.05$) increased compared with the control by the application of azoxystrobin. Additionally, activities of superoxide dismutase, catalase, ascorbate peroxidase, peroxidase, and ginsenoside contents in azoxystrobin-treated plants were improved, and malondialdehyde content and O_2^- contents were reduced effectively. Azoxystrobin treatments to ginseng plants at all growth stages suggested that the azoxystrobin-induced delay of senescence was due to an enhanced antioxidant enzyme activity protecting the plants from harmful active oxygen species. When the dose of azoxystrobin was 225 g a.i./ha, the effect was more significant.

Conclusion: This work suggested that azoxystrobin played a role in delaying senescence by changing physiological and biochemical indices and improving ginsenoside contents in ginseng leaves.

1. Introduction

Azoxystrobin is the name of the compound methyl (E)-2-{2[6-(2-cyanophenoxy) pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate [1]. Azoxystrobin retains the methyl β-methoxyacrylate group of the naturally occurring strobilurins, by inhibiting mitochondrial respiration by blocking the transfer of electrons in the mitochondrial b,c1 complex for mitochondrial electrons [2,3]. Strobilurins are first discovered from wood-decaying *Basidiomycete* species [4]. Strobilurins have been proved good in yield increase and improving the quality of agricultural produce [5,6]. In earlier studies, wheat treated with azoxystrobin showed significant increases in production [7,8]. Enhanced postharvest, delayed senescence, and water conservation are some of the positive physiological changes of strobilurin treatment that have been reported [9–12].

Ginseng (*Panax ginseng* Meyer) is a valuable medicinal plant that has been used extensively in many countries for more than 5,000 yr [13]. Ginseng is known for its ginsenosides that have ecological and pharmacological benefits [14]. A large number of ginsenosides are a major constituent found in different tissues of ginseng [15,16]. Ginseng is generally harvested after a cultivation period of >4 yr [17,18]. Studies have shown that the contents of these active components in ginseng increase every year during the fast-growth period and remain relatively stable during the slow-growth period [19,20].

Peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) are widely distributed enzymes in plants [21]. As we all know, leaf senescence is an important oxidative process [22]. Active oxygen is involved in biochemical and physiological changes during leaf senescence [23]. The enzymatic antioxidant system is a protective mechanism [24]. Nowadays, many research works have

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revealed the reaction pattern (alkaline stress, senescence, acid stress, and salt stress) [25–28]. Strobilurin fungicides have been shown to have antioxidative properties [29]. Most of these investigations focused on the effect of azoxystrobin [30].

Azoxystrobin is a systemic fungicide. We investigated its impact on the physiological and biochemical indices, and ginsenoside contents of ginseng leaves.

2. Materials and methods

2.1. Plant material and azoxystrobin application

During the growing season of 2013, ginseng (*Panax ginseng* Mey. cv. "Ermaya") was grown on Baishan Experimental Farmland [Experimental Farmland of Jilin Province in China (E126°18′, N41°42′; henceforth abbreviated as "BS"] and Huanren Experimental Farmland [Experimental Farmland of Liaoning Province in China (E124°47′, N41°32′); henceforth abbreviated as "HR"). When plants were at the phenological growth stage ([PGS]) 800/909 (all fruits green) [31], 5-yr-old ginseng plants were chosen and divided into three plots (each plot 15 m^2 in size). At [PGS] 800/909 (all fruits green), 25% azoxystrobin SC plus water control were sprayed. The application equipment was a 3WBD-16 power sprayer. Air temperature at azoxystrobin SC application was 27.2–29.1°C, and the weather was sunny or cloudy. Wind velocity was 0.5–0.7 m/s. azoxystrobin SC (25%) was sprayed three times at 7 d intervals. The following azoxystrobin doses were used: 25% azoxystrobin SC 150 g a.i./ha and 225 g a.i./ha. The experiment was repeated in the same fields, but not in the same plots, in the growing season of 2014.

2.2. Sampling preparation

At [PGS] 801/909 (beginning of fruit reddening), [PGS] 805/909 (50% of fruit red), [PGS] 809/909 (fruit fully ripe), [PGS] 902/909 (beginning of leaf yellowing), and [PGS] 903/909 (most of the leaves yellowish and drooping), five samples of ginseng were taken from each plot every time. One part of leaves was frozen in liquid N_2 and stored at −86°C in a refrigerator (cryogenic, DW-HL388) until extraction for enzyme analyses. The other part of leaves was used for chlorophyll (CHL), soluble protein (SP), malondialdehyde (MDA), superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and ginsenoside content assays immediately.

2.3. Measurement of CHL and SP contents

CHL was extracted from leaf blade discs (3 per replication), by homogenizing in acetone and ethanol solution (1:1, v:v); absorbance of the leaf extract was measured at 664 nm [10] using a spectrophotometer (Shimadzu UV-2450). SP content was assayed by the method of Bradford [32] using bovine serum albumin as a standard and expressed as mg/g FW. Absorbance of the sample was measured using a spectrophotometer.

2.4. Measurement of H_2O_2, O_2^-, and MDA contents

H_2O_2 was assayed following the modified method of Bonasia et al [10]. The absorbance was immediately measured at 450 nm using a spectrophotometer. The O_2^- assay followed the modified method of Lu et al [33]. The absorbance was measured at 530 nm using a spectrophotometer. MDA content was measured by the TBA method and expressed as μmol/g FW [34].

2.5. Measurement of antioxidative enzyme activity

SOD activity was determined according to the modified method of Ferreira et al [35]. SOD activity was expressed as U/g FW, and measured at 560 nm by a spectrophotometer. POD activity was assayed by measuring the oxidation of guaiac-based phenol in the presence of H_2O_2 [36]. It was defined as an absorbance change of 0.01 in 1 min at 470 nm using a spectrophotometer. CAT activity was determined using the method of Zhang et al [7]. It was followed by measuring the absorbance change at 240 nm using a spectrophotometer. Ascorbate peroxidase (APX) activity was measured by the method of Rahman and Punja [37], which was measuring the absorbance change at 290 nm using a spectrophotometer. Every index was determined three times per replication.

2.6. Extraction and analysis of ginsenosides

Extraction of ginsenosides was based on the method described by Palazón et al [38]. Extracts of ginsenosides were analyzed by HPLC (Shimadzu LC20-AB) utilizing a GL-Wondasil C_{18} (250×4.6 mm $\times$ 5 μm) column. Ginsenosides Rb_1 (98.5%), Re (98.5%), and Rg_1 (98.5%) were purchased from Jilin University in China.

2.7. Statistical analysis

Statistical analysis was carried out with the analysis of statistically significant (SPSS for Windows, version 18.0) and means were separated with the least significant difference test at $p = 0.05$, to determine whether azoxystrobin had a significant effect on leaf senescence and ginseng ginsenoside contents [39].

3. Results

Similar results were recorded for each dose treatment or water control from the two experiments in 2013 and 2014. Statistical analysis revealed that there was no significant difference between the two sets of data ($p = 0.05$). Thus, data from the two experiments were combined.

3.1. Effect of azoxystrobin on CHL and SP contents

The overall levels of CHL content in ginseng leaves increased until [PGS] 809/909 and then declined with further aging of plants (Fig. 1A). It showed that CHL contents of azoxystrobin-treated plants were higher than those of the control plants at all growth stages in both BS and HR of 150 g a.i./ha and 225 g a.i./ha. On the 3rd day of [PGS] 809/909 (fruit fully ripe), CHL contents of 150 g a.i./ha azoxystrobin-treated plants were 1.26 times those of the control plants in BS and 1.47 times in HR, and CHL content of 225 g a.i./ha azoxystrobin-treated plants was 1.53 times higher than that of the control plants in BS and 1.63 times higher in HR. At [PGS] 903/909, CHL contents of 150 g a.i./ha azoxystrobin-treated plants were 2.2 times those of the control plants in BS and 1.5 times in HR, and CHL content of 225 g a.i./ha azoxystrobin-treated plants was 2.58 times higher than that of the control plants in BS and 2.68 times higher in HR. Thus, CHL content was higher with the application of 25% azoxystrobin SC 225 g a.i./ha than with 150 g a.i./ha, and it was higher in BS than in HR. Moreover, at [PGS] 903/909, CHL contents of azoxystrobin-treated plants were higher than those of the control plants at [PGS] 902/909 in both BS and HR. It suggested that azoxystrobin played a role in improving CHL contents in ginseng leaves; the leaves of ginseng treated with 225 g a.i./ha azoxystrobin reached 903/909 at 120 d after 800/909, while the control reached at 140 d.

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