



Reduced phytotoxicity of propazine on wheat, maize and rapeseed by salicylic acid



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ABSTRACT

Propazine belongs to the triazine herbicide family and widely used in the farmland for crop production. Recent studies have shown that the residue of propazine in environment is accumulative. This inevitably results in accumulation of propazine in crops. Therefore, reduction of propazine toxicity and accumulation in crops is critically important. In this study, the growth of wheat, maize and rapeseed was significantly inhibited by 2, 8 and 0.4 mg kg⁻¹ propazine in soils. The chlorophyll content of the three crops also showed significant decrease, while the electrolyte permeability, a biomarker of cellular damage, increased in the plant cells. However, when plants were sprayed with 5 mg L⁻¹ of salicylic acid (SA), the propazine phytotoxicity of the crops was relieved, with increased chlorophyll content and reduced electrolyte permeability of all crops. Meanwhile, the activities of peroxidase (POD) and glutathione transferase (GST) remained lower. The propazine accumulation in the crops and the residues in the soil were determined by high performance liquid chromatography. The concentration of propazine in plants and soils treated by SA was less than that of the untreated control. Six propazine degraded products (derivatives) in rhizosphere of wheat were characterized using ultraperformance liquid chromatography with a quadrupole-time-of-flight tandem mass spectrometer. Our work indicates that the improved growth of crops was possibly due to the acceleration of propazine degradation by salicylic acid.

1. Introduction

Propazine [2-chloro-4,6-bis (isopropylamino)-s- triazine] is a herbicide widely used for controlling weeds in agricultural area (Rutherford and Krieger-Liszskay, 2001). The long-term input of propazine in the farmland leads to a series of environmental problems. One of them is the pollutive effect on crop production (Keeling et al., 2011). As propazine is structurally stable, its half-life is as long as more than several months (Jiang et al., 2017). Apparently, it is environmentally resistant. Propazine has been frequently detected in the soil surface and groundwater (Topp et al., 2000) and even in some birds and mammals (Reindl et al., 2015; Koroša and Auersperger, 2016). Therefore, it is important to find a way of degrading and detoxifying propazine in crops growing in the herbicide-polluted soil.

Salicylic acid is a class of plant regulators that play multiple roles in plant development as well biotic and abiotic stress responses (Catinot et al., 2008). Numerous studies have shown that SA acts as a signal

transduction molecule participating in the systemic resistance to improve the capability of coping with environmental stresses (e.g. heat, drought, salt and heavy metals) (Wang et al., 2004; Mutlu et al., 2009; Liang et al., 2012; Wang and Zhang, 2017). Some reports indicate that pesticide-induced phytotoxicity and cellular damage can be alleviated by SA (Liang et al., 2012; Lu et al., 2015a). Wheat seedlings planted in the soils containing 4 mg kg⁻¹ isoproturon and followed by SA spraying showed improved growth and physiological responses (Liang et al., 2012). SA was also shown to lower the toxic effect of high concentration chlorpyrifos (≥ 20 mg kg⁻¹) on wheat by reducing chlorpyrifos accumulation in plants (Wang and Zhang, 2017). However, the mechanism for SA mediating plant toxic response to herbicides is not fully understood.

When plants are exposed to excessive pesticides, the reactive oxygen species (ROS) are massively generated (Song et al., 2007). The excessive ROS jeopardize cellular molecules, damage physiological process, and eventually lead plants to death (Steduto et al., 2000).

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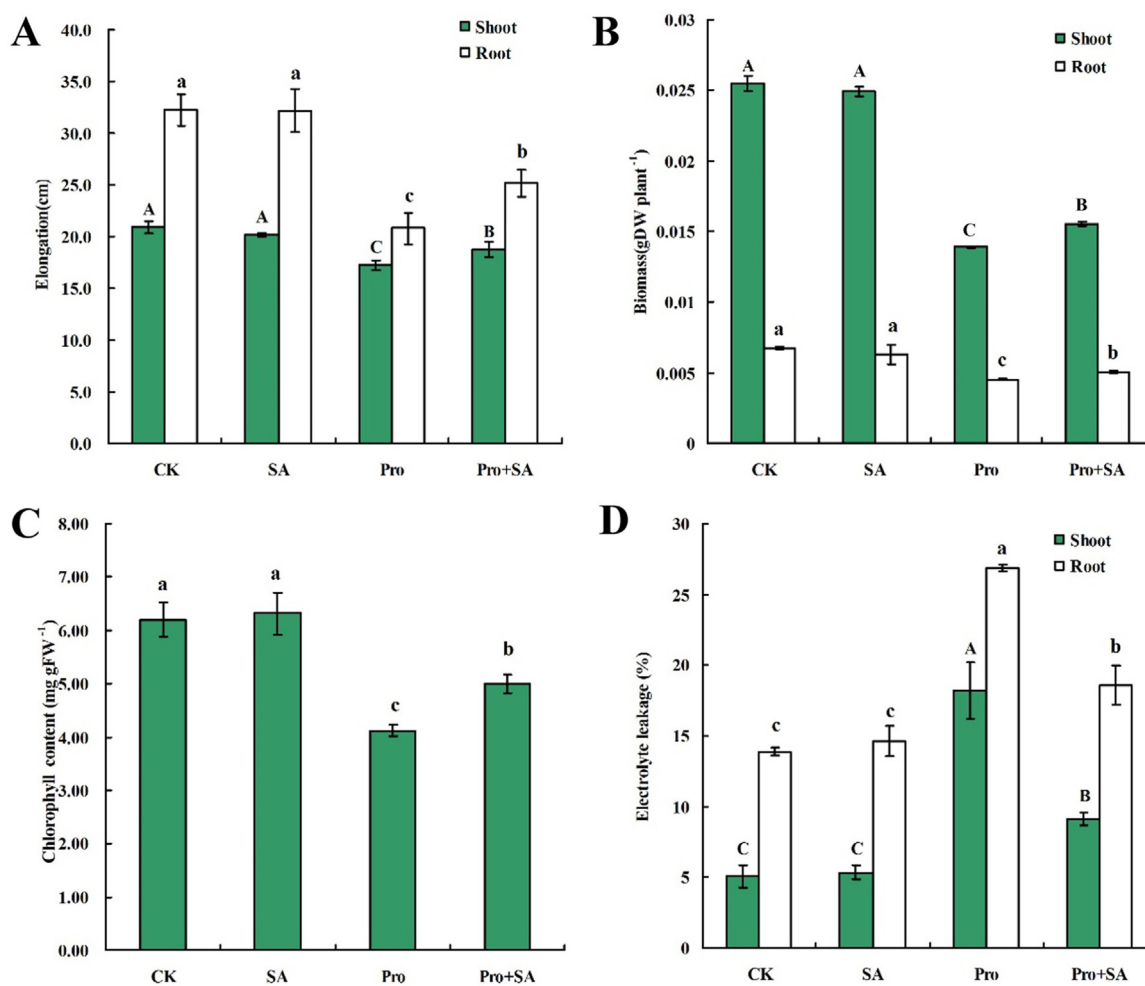


Fig. 1. Effect of propazine (Pro) and/or SA on growth of wheat. A, Elongation; B, Biomass; C, Chlorophyll content; D, Electrolyte leakage. Pro: 2 mg kg⁻¹ only; and SA + Pro: Pro (2 mg kg⁻¹) + SA (5 mg L⁻¹). Values are the means \pm SD ($n = 3$). Data with the different letter are significantly different ($p < 0.05$).

Peroxidase (POD) is an active antioxidant enzyme in plants, catalyzing the removal of hydrogen peroxide by oxidizing its target substances such as phenols or other metabolites and thereby eliminating ROS-triggered damage to the cells (Yin et al., 2008). POD is considered as a biomarker involved in plant resistance to many environmental stresses (Zhang et al., 2011). Glutathione *S*-transferase (GST) is one of most important metabolic enzymes catalyzing conjugation of endogenous or exogenous harmful electrophilic groups with reduced glutathione and making it easier detoxified in plant cells (Jiang and Yang, 2009). Understanding how POD and GST activities are mediated by SA under propazine exposure may help figure out the mechanism underlying the detoxification and degradation of propazine in plants.

More than 80% of herbicide residues remain in soils after field administration (Fang et al., 2009). Soil microorganisms play a pivotal role in degrading toxic organic chemicals including herbicide propazine. For example, the red bacteria tend to degrade propazine in the way of dealkylation (Mills and Thurman, 1994). Nocardia strain AN4-4 degrades propazine through hydroxylation of dechlorination metabolites (Satsuma, 2009). Pseudomonas strain can degrade the triazine herbicides through dechlorination forming hydroxyl methylation product (Seffernick et al., 2000). The microbial-based degradation of pesticides is largely facilitated by plant growth in soils, because the exudates and residues of roots are the major sources for microbial survival (Wei et al., 2015). Importantly, the degradation of pesticides could be promoted by SA (Liang et al., 2012). Supplying plants with SA lowered isoproturon residues in rhizosphere and enhanced root exudation of organic acids; meanwhile, the microbial population and soil enzyme activities

associated with soil activity in rhizosphere were activated (Lu et al., 2015a).

Wheat, maize and rapeseed are representative of the most important crops worldwide. They provide major calories and food oils for human diet. Investigating the propazine residues in the crops is critically important to ensure the crop production and food safety. Thus, the goal of the study is to figure out how the propazine residues would be detoxified and degraded by SA. This study will help understand a role of SA in reducing toxicity and damage of propazine to crops and mechanism for accelerating propazine degradation in the crops and soils.

2. Materials and methods

2.1. Plant growth and treatment

Propazine (purity of 97%) was provided by the Institute of Pesticide Science, Academy of Agricultural Sciences in Jiang Su, Nanjing, China. Propazine-free soil was collected from the surface soil of Nanjing Agricultural University Station, with major chemical properties of organic carbon, 2.13%; total N, 1.26 g kg⁻¹; available P, 34.3 mg kg⁻¹ and available K, 91.5 mg kg⁻¹; pH 7.6. The collected soil was air-dried, crushed, and passed through a 3 mm sieve (Sui and Yang, 2013). Seeds of wheat (Yangmai 13), maize (Jiangnan waxy) and rapeseed (Nannong 2) were surface-sterilized and germinated in darkness at 25 °C for 24 h. The germinating seeds were transferred to a plastic pot (500 mL) containing 430 g soil. Seedlings were grown in a growth chamber under the condition of a light intensity of 300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, a light/dark

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