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Mechanical Wounding Induces Catalase Gene (*VsCat*) in Leaves of Common Vetch

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ABSTRACT

Background: In plants, wounding can result from mechanical injuries or from biotic factors induced by herbivores infestation and pathogen infection. Wounding enhances the production of reactive oxygen species (ROS). Enzymatic and nonenzymatic defense systems have been reported in plants to immediately combat increased levels of ROS. Plant catalases are encoded by a multigene family and are the major scavenging enzymes catalyzing the dismutation of toxic hydrogen peroxide to water and dioxygen.

Methods: In the preasent work, a quantitative real-time PCR was used to quantify the expression level of a catalase gene from common vetch (*Vicia sativa*; *VsCat*) in response to mechanical wounding.

Result: The results of the time course study showed that the transcript levels of *VsCat* were significantly increased in wounded leaves at all-time points examined with a peak expression of 7.6 folds at 2 h post wounding. The increased expression of *VsCat* might represent a direct defense against elevated H_2O_2 generated during wounding.

Keywords: Enzymatic defense mechanism, Gene expression, Hydrogen peroxide, *Vicia sativa*.

INTRODUCTION

During their life cycle, plants are frequently exposed to different biotic and abiotic stress factors (Abu-Romman, 2016a; Salman *et al*., 2017; Abid *et al*., 2020). These stresses lead to a series of molecular and metabolic responses, adversely affecting crop growth, development, and productivity (Al-Momany and Abu-Romman, 2014; Ahanger *et al*., 2017; Sadder *et al*., 2019).

Under normal growth conditions, reactive oxygen species (ROS) are normally and continuously produced at minimal levels in different subcellular compartments. However, environmental stresses enhance the production threshold of ROS to the level that imposes oxidative damages to membranes and macromolecules (Apel and Hirt, 2004; Gill and Tuteja, 2010; Farooq *et al*., 2019). Under stress conditions, enzymatic and non-enzymatic scavenging mechanisms are operating to control elevated ROS levels. Superoxide dismutase, ascorbate peroxidase, catalase, and glutathione reductase are the major detoxifying enzymes to combat ROS (Abu-Romman and Shatnawi, 2011). Whereas, the main non-enzymatic antioxidants are ascorbate and glutathione (Basu *et al*., 2010).

Leaf wounding can result from biotic factors by herbivores infestation and pathogen infection, or it can result from abiotic means in the form of mechanical injury like heavy precipitation, wind and harvesting (León *et al*., 2001). Wound responses in plants involve the activating of multiple signaling pathways responsible for healing and defense-responsive genes (Wasternack *et al*., 2006). ROS were reported to rapidly accumulate in wounded leaves, and play a role in signaling (Suzuki and Mittler, 2012; Al-Momany and Abu-Romman, 2016; Lew *et al*., 2020).

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Catalase (E.C. 1.11.1.6) is the major scavenging enzyme which catalyzes the dismutation of toxic H_2O_2 to water and dioxygen (Asada, 1999). These heme containing enzymes are mainly peroxisomal and unlike animals, catalases in plants are encoded by a multigene family (Kamigaki *et al*., 2003; Mhamdi *et al*., 2010). Different catalase classes possess different expression patterns in different organs and in response to stresses (Purev et al., 2010; Su *et al*., 2014; Sun *et al*., 2018). A catalase gene was cloned and characterized from the forage legume common vetch (*Vicia sativa*), and this gene was up-regulated under different abiotic stresses and hormonal stimuli (Abu-Romman, 2016b). The molecular responses of this gene is not yet investigated in wounded leaves of common vetch. Therefore, the present study aims at investigating the expression pattern of catalase gene (*VsCat*) in response to wounding in common vetch.

MATERIALS AND METHODS

Plant material and wounding

Seeds of common vetch (*Vicia sativa* cv. Mahali) were planted in a greenhouse as previously described (Abu-Romman, 2019). Mechanical wounding was carried out on intact three-week old common vetch plants by wounding 6 leaves per plant using scissors without damaging the main leaf vein. Collected tissues from wounded and unwounded leaves were quickly frozen in liquid nitrogen.

RNA isolation and VsCat expression

Total RNA was extracted from wounded leaves using Total RNA Purification Kit (Jena Bioscience, Germany), and cDNA from was synthesized from 3 µg of total RNA using PrimeScript™ RT Master Mix (TaKaRa, Japan).

The relative expression pattern of *VsCat* was explored in wounded leaves by quantitative real-time PCR (qRT-PCR) using KAPA SYBR® FAST qPCR Kit (KAPA BIO, USA). Primers used in qRT-PCR to amplify *VsCat* (forward: 5'-ATCCCCAGACTCACATCCAGG-3'; reverse: 5'-TTTCCCAGCCTTGTTGAGCAG-3') were designed based on *VsCat* coding sequence available in GenBank (Accession No. KX090583.1). Data of expression analysis were presented as fold change of *VsCat* transcripts normalized to *V. sativa Actin* (*VsAct*) reference gene (Accession No. GU946218) and set relative to that in unwounded plants, using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008). Primers used to amplify *VsAct* (forward: 5'-CAATCCAGGCCGTCTTGTCTC-3'; reverse: 5'-TCTGTTAAATCACGCCCAGCA-3') yield 157 bp amplicon. Results were expressed as means of three biological replicates and were statistically analyzed using LSD.

RESULTS AND DISCUSSION

Environmental stresses induce a drastic accumulation of reactive oxygen species (ROS) in different cellular compartments. These ROS cause oxidative damages to the major cellular macromolecules (Apel and Hirt, 2004; Gill and Tuteja, 2010; Farooq et al., 2019). Defense systems have been evolved in plants to immediately combat increased levels of ROS. Enzymatic and nonenzymatic antioxidants detoxify ROS in different organelles (Basu *et al*., 2010; Abu-Romman and Shatnawi, 2011). In this work, expression analysis was performed using qRT-PCR to explore the time course changes of transcript levels of *Cat* gene following mechanical wounding in common vetch.

Transcript levels of *VsCat* were significantly increased in wounded leaves at all-time points examined (Fig 1). The expression of *VsCat* was gradually up-regulated and scored 3.4- and 5.7-fold increase at 0.5 and 1 h post wounding, respectively. Afterward, the expression level further increased and reached a peak of 7.6 folds at 2 h post wounding treatment. On the other hand, drops in *VsCat* expression were recorded at 4 and 6 h of treatment and reached 3.9- and 3-fold, respectively. In plants, wounding caused from abiotic and biotic factors and induces the generation of ROS (León *et al*., 2001; Maffei *et al*., 2007). The increase in transcript levels of *VsCat* might represent a direct defense against elevated H_2O_2 generated during wounding. Moreover, different studies have shown increased levels of JA biosynthesis in wounded plants (Nyathi and Baker, 2006; Chen *et al*., 2019), which might contribute in increasing *VsCat* expression. In accordance to the present observation, different studies have showed increased *Cat* expression in response to wounding-associated H_2O_2 accumulation (Guan and Scandalios, 2000; Dong *et al*., 2015). Taking into account that *VsCat* encodes a peroxisomal Cat enzyme (Abu-Romman, 2016b), wounding was reported to induce the expression of peroxisome biogenesis genes (Lopez-Huertas *et al*., 2000).

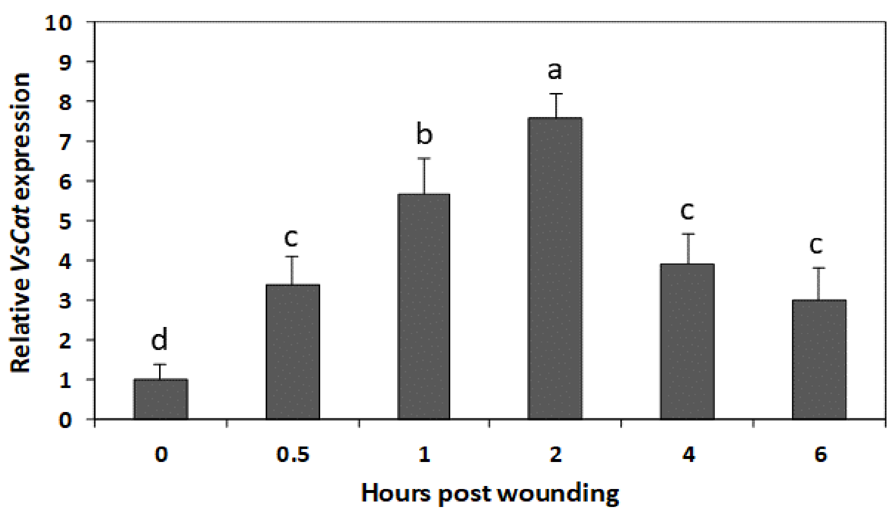

Fig 1: Expression analysis of *CAT* in response to wounding in *Vicia sativa*. Transcript levels in *V. sativa* wounded leaves were quantified using qRT-PCR. The expression of *VsCAT* at each time point was normalized to the *Actin* gene (internal control) and expressed as a ratio relative to unwounded plants (0 h; set at 1). Each mean value represents the averages of three replicates. Bars represent standard errors and means with different letters are statistically different ($p \leq 0.5$) according to LSD.

Contributions

Saeid Abu-Romman and Tarek Ammari: Experimental design and article drafting.
Saeid Abu-Romman: Experimental operation.

Declaration of Competing Interest

I declare that the publication of this paper does not involve any conflict of interest between individuals or groups.

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