Tropical Plant Pathology, vol. 36, 3, 169-177 (2011)

www.sbfito.com.br

RESEARCH ARTICLE / *ARTIGO*

Analysis of tomato seedling cell death in response to copper and paraquat induction

Antoni Rueda[1], Yony Roman[1], Mario Lobo[2] & Carlos Pelaez[1]

[1]Universidad de Antioquia, Medellín, Colombia; [2]Corpoica, Rionegro, Antioquia, Colombia

Author for correspondence: Carlos Pelaez, e-mail: cpelaez@matematicas.udea.edu.co

ABSTRACT

Plant defense responses to stress have attracted interest because of their similarity to reported mammalian stress responses. To investigate plant responses to abiotic stress, caspase 10-like enzymatic activities of *Lycopersicon hirsutum* and *Lycopersicon esculentum* were assayed in response to *copper chloride* and paraquat induction. Caspase 10-like activity was greatest at 40 mM $CuCl_2$ at 9 h after elicitation in both *L. hirsutum* and *L. esculentum,* for which respective response slopes (Δ absorbance per Δ minute) were 0.0054 and 0.0022 . The response for *L. hirsutum* was less variable and significantly greater than for *L. esculentum.* Elicitation of caspase 10-like activity by paraquat was greatest at around 2 h after treatment in both species with slopes of 0.013 and 0.0012, respectively for *L. hirsutum* and *L. esculentum.* The direct determination of caspase 10-like activity in tomato seedlings treated with copper and paraquat, using a specific substrate for mammals, suggested that the observed responses were related to apoptosis processes under abiotic induction.
Key words: *Lycopersicon*, abiotic elicitation, apoptosis, caspase activity

RESUMO

Analise da morte cellular das plântulas do tomate na resposta à indução com cobre e paraquat

A pesquisa relacionada com a resposta inata das plantas á estresses tem atraído muito interesse por a similitude com as respostas reportadas nos mamíferos. Assim, foi proposta uma aproximação ás respostas de defesa aos estresses abióticos através da avaliação no modelo biológico *Lycopersicon spp.* da atividade enzimática caspase 10 em resposta da indução com $CuCl_2$ y paraquat. Os ensaios da atividade caspase 10 mostraram um máximo de atividade na concentração 40 mM, 9 horas depois da elicitação, com uma pendente de 0,0054 e 0,0022 para *L. hirsutum* y *L. esculentum* respectivamente. A atividade comparativa dos genótipos sugere que, embora os dois genótipos presenteassem atividade, a resposta é menos variável de maior magnitude para *L. hirsutum.* A elicitação com paraquat presenteou a maior atividade caspase 10 em torno das 2 horas depois do estímulo, com uma pendente de 0,013 e 0,0012 para *L. hirsutum* y *L. esculentum* respectivamente. A determinação direita da atividade caspase 10 nas plântulas de tomate tratadas com cobre e paraquat, usando um substrato específico para mamíferos, mostrou que estes mecanismos estão relacionados provavelmente com processos de apoptose depois da indução abiótica.
Palavras-chave: *Lycopersicon*, apoptose, atividade de caspase, eliciação abiótica.

INTRODUCTION

Stress involves the presence of an external factor that exerts a negative influence on the optimal development of the plant (Azcon-Bieto & Talon, 2003); the stress can be biotic or abiotic (Hahn, 1996; Hammerschmidt, 1999; Grayer & Kokubun, 2001; Zhaoa et al., 2005). Abiotic factors, such as heavy metals, xenobiotic compounds, and herbicides, contribute to adverse effects on plants. Although copper is an essential microelement for plants, it has toxic effects in high concentrations (Burda et al., 2002). The metal has been proposed as an inhibitor of the electron supply chain in the primary plastoquinone A acceptor (QA) and pheophytins and in the secondary plastoquinone B acceptor (QB). It has also been suggested that copper inhibits plant photosystem processes through tyrosine residue (Tyrz) oxidation (Yruela et al, 1996; Burda et al., 2002; Ke, 2007). Paraquat (1,1´-dimethyl-4,4´-bipyridinium dichloride) was reported to act by diverting electrons to the supply chain in photosystem I, especially during the transit of electrons from the cysteine sulfur complex toward ferredoxin, thus interrupting the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH In addition, copper and paraquat have been associated with an oxidative burst, which theoretically triggers structural damage to cells (Mithofer, 2004). Despite these frequently reported mechanisms, detailed studies of other possible mechanisms leading to cell death have not been reported. In this research new aspects regarding the action mechanisms of these elicitors are proposed.

Abbreviations: HR - hypersensitivity response; PCD - programmed cell death; ROS - reactive oxygen species.

In recent years, a parallel has been drawn between the apoptotic processes in mammals and the hypersensitive reactions in plants (Danon et al., 2000; Godbole et al., 2003; Hofius, 2007). Functionally and mechanistically, two forms of cell death have been recognized. In animals, apoptotic and necrotic death have been defined using morphological, biochemical and, more recently, molecular criteria. Necrosis is a mechanism of cell death that involves swelling of cells and organelles, followed by plasma membrane rupture and release of cytoplasmic material (Golstein et al., 2003). Apoptosis was originally defined in 1972 based on specific morphological changes that occur during a genetically controlled cell death initiated by environmental stimuli (Kerr et al., 1972). In a simplified view, apoptosis is associated with chromatin condensation, cell shrinkage, plasma membrane blebbing, internucleosomal DNA cleavage, the formation of apoptotic bodies and finally the engulfment and degradation in phagocytic lysosomes of adjacent cells (Clarke & Clarke, 1995). Each newly discovered step was successively incorporated in a cell suicide pathway referred to as programmed cell death (PCD), which implies the activation of genome-encoded biochemical pathways and is part of the life cycle of multicellular animals and plants. By controlling cell numbers and turnover, forming and deleting structures, and eliminating damaged or infected cells, PCD is critical to normal development and immune responses (Baehrecke, 2002). Different meanings have been ascribed to the terms apoptosis and PCD (Vaux, 1999). For example, some researchers restrict apoptosis to the morphological phenotype observed in some forms of animal PCD and for others PCD means developmental cell death. In the present research, apoptosis is considered from the biochemical point of view, *differentiating* it from PCD, for which a genome-encoded biochemical pathway has been shown to lead to cell destruction.

A critical question in the study of cell death in plants is the extent to which hallmarks of apoptosis and of animal PCD can be found. Core components of the apoptotic machinery are caspases, a family of cysteine proteases. Caspases are activated by extrinsic death receptor pathways, or by intrinsic pathways that involve mitochondrial and endoplasmic reticulum (ER) release of proapoptotic factors, and the regulatory functions of BCL-2 protein family members (Hengartner, 2000). The caspases constitute a family of cysteine proteases that use a cysteine residue as the catalytic nucleophile and that share an exquisite specificity for cleaving target proteins at sites next to aspartic acid residues (Boatright & Salvesen, 2003). Caspases are essentially proteases with a cysteine residue acting as the catalytic amino acid (Earnshaw et al., 1999; Yan & Shi, 2005). Although plant caspase genes have not yet been identified, their existence has been indicated by many studies in which caspase inhibitors block the hypersensitive response (HR) and other defense mechanisms (Danon, 2000). Recently, treatment of tomato cell suspensions with $AlCl_3$ was reported to induce programmed cell death (PCD), a conclusion reached based on elicitation of the cells and treatment of the cells with different caspase inhibitors (Yakimova et al., 2007). In another work, specific peptide inhibitors of AC-YVAD-CMK and AC-DEVD-CHO caspases led to suppressed bacteria-induced PCD in tobacco leaves (Del Pozo & Lam, 1998). In addition, an apoptosis system in free cells of oats induced with victorin toxin exhibited apoptosis, nuclear collapse and DNA fragmentation in isolated nuclei (Kusaka et al., 2004). The aim of the present work was to study the responses of *Lycopersicon* spp. by evaluating the caspase 10-like enzymatic activities in response to $CuCl_2$ and paraquat induction.

MATERIALS AND METHODS

Genetic resource

Two tomato species were used: the wild-type species *Lycopersicon hirsutum* (PI 251305), which was reported to be resistant to several plant pathogens, and the cultivated species *Lycopersicon esculentum* (L-507, Licato type). These plants were obtained from the Sistema de Bancos de Germoplasma Vegetal de la Nación Colombiana (Colombian National Plant Germplasm Bank System), which is managed by the Corporación Colombiana de Investigación Agropecuaria, Corpoica (Colombian Corporation for Agricultural Research).

Regeneration and Material Propagation

The tomato seeds were sown in a sterile environment with nutrients. After germination, the seedlings were transplanted into trays of sterile soil with controlled water supply in a greenhouse with natural light, and later to flower pots in which they were allowed to develop to the reproductive phase.

Elicitation and evaluation of ex situ materials

Young leaflets of *Lycopersicon hirsutum* and *Lycopersicon esculentum* seedlings were sprayed with solutions of 40 mM $CuCl_2$ or 1% paraquat. For assays of caspase 10-like activity, leaflets of 4 seedlings (experimental unit) were harvested at 0, 2.5, 5.5, 8.5, 15 and 24 h after elicitation. The kinetics of the caspase 10-like activity was investigated for two replicates at each time of sampling. The harvested leaflets were weighed and immediately macerated at -70°C (–94°F) for storage and later use, or at 20°C (-4°F) for caspase 10-like activity assays. Two repetitions of each caspase 10 experiment were conducted.

Measurement of caspase 10-like activity

An extraction buffer to obtain the enzymes from the frozen leaves was prepared with 250 mM NaCl, 50 mM $NaH_2PO_4.H_2O$, 10% glycerol, 1 mM EDTA, 1% BSA, and 1 mM DTT at pH 8.0 (the extraction buffer was optimized from the proteolytic activity measured at different pH values). The caspase 10-like activity was measured using a

kinetic method with N-Acetyl-Ala-Glu-Val-Asp-7-amido-4-trifluoromethyl coumarin, which is a specific substrate for caspases. To assay the enzymatic kinetics, the reaction was stopped at 0, 2, 4, 6, 8, 10, 15, 20 and 25 min in a reaction consisting of a 1:1 ratio of 25 µL of the enzymatic reactions and 25 µL of the stop solution composed of 1% CH_3COONa and 4.5 mM CH_3COOH at pH 4.5. The reaction was read at 380 nm. The enzymatic activity was determined as the initial velocity (slope) and is expressed as follows: Δ absorbance per Δ min of the catalytic product from the reaction of 0.075 µmoles of substrate with 125 µL of extract, which was obtained from the macerated sample (1:2 mg leaf tissue:µL of buffer). Additionally, dose-response assays were developed using concentrations of 10, 40 and 80 mM $CuCl_2$ and 3, 1 and 0.25 % paraquat.

Analysis of the results

To analyze the caspase 10-like activity data, linear regressions were conducted for each time point (Statgraphics Centurion XV). The slope for each sampling point (enzymatic activity) was used to construct a graph as a function of time after the elicitation. To determine the reproducibility of the caspase assays, variance analyses were conducted with the SAS statistical package version 8.0 for independent-in-time assays. To this end, analyses were done under the completely randomized model arrangement.

RESULTS

Caspase 10-like activity assays during copper elicitation

For use of the extraction buffer in assays, its pH was optimized in relation to enzyme activity (Figure 1). The effects of seedling induction with copper and paraquat on caspase 10-like activity of *L. hirsutum* and *L. esculentum* were assessed. Seedling elicitation of both species with a 40 mM $CuCl_2$ solution showed caspase 10-like activities at 3, 6 and 9 h after elicitation (Figure 3). Observations for the copper-treated seedlings indicated that a process of physiological death took place during the experiment (Figure 2). This process can be associated with caspase 10-like activity which was about three times greater for *L. hirsutum* than for *L. esculentum* (Figures 3-4).

Dose-response effect of caspase 10-like activity during copper elicitation

The dose effect was assessed 9 h after elicitation, when the highest level of activity was observed for copper elicitation. For these assays, effects of 10, 40 and 80 mM $CuCl_2$ were measured in two independent experiments. A dose-response effect was evident for both tomato species. In *L. hirsutum* the level of activity was higher for the 40 mM concentration than for 80 mM, and no activity was observed for the 10 mM concentration (Figure 5). The dose-response behavior of *L. esculentum* differed from that of *L. hirsutum*. While the highest level of activity was observed for the 40 mM concentration, considerable activity was observed for the10 mM dose, and no activity was observed for the 80 mM concentration (Figure 6). The dose-response assays revealed that the 40 mM dose used during the kinetic experiments was adequate to induce caspase 10-like activity.

Caspase 10-like activity during paraquat elicitation

The elicitation with paraquat was performed in two independent time-course assays, which revealed activity for both tomato species (Figures 7-8). A comparison between the activity of the taxa showed differences in favor of *L. hirsutum*, which exhibited a maximum caspase 10-like activity around 2.5 h compared to *L. esculentum.* Nevertheless, the response was much higher in magnitude for *L. hirsutum*, which suggested that the mechanisms associated with apoptosis were activated to a greater extent for this species. These results isuggest events associated with apoptosis, rather than an exclusive cell damage sequence as a result of an oxidative burst.

Dose-response effect of caspase 10-like activity during paraquat elicitation

Dose-response effects were measured at 3 h after paraquat elicitation when the level of caspase 10-like activity was greatest. Two independent experiments were conducted in which the concentrations of 0.25, 1 and 3% paraquat were used. The responses were similar for the two

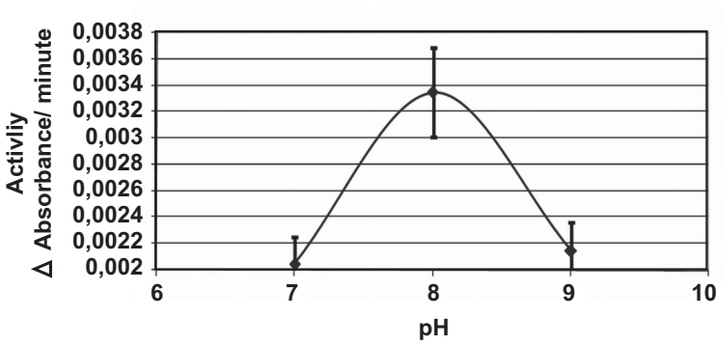

FIGURE 1 - pH optimization of the extraction buffer in relation to the proteolytic activity. The error bars corresponded to the standard deviation of two different time-independent assays.

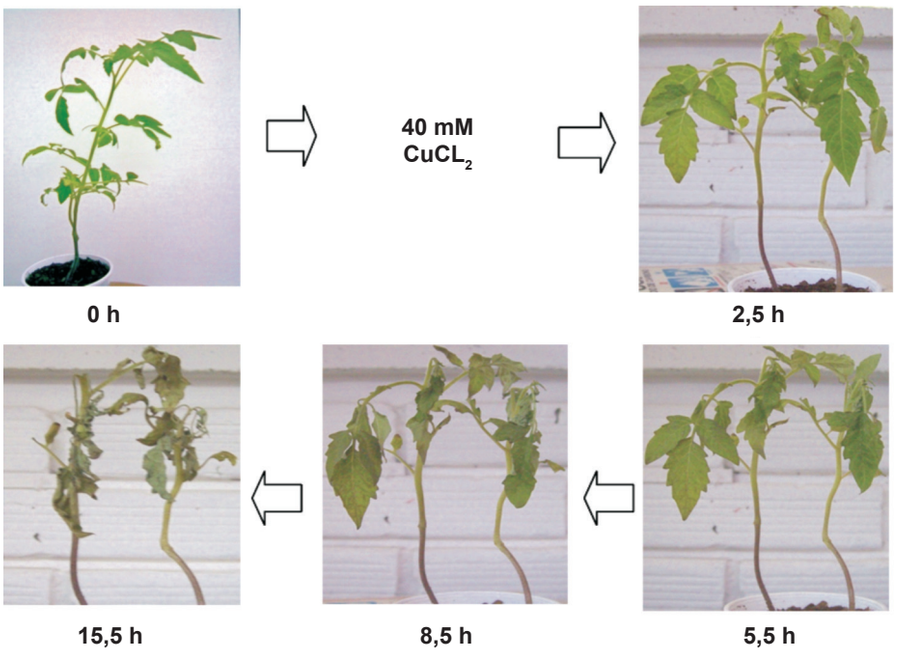

FIGURE 2 - Kinetics of death of *Lycopersicon esculentum* seedlings treated with $CuCl_2$ 40 mM

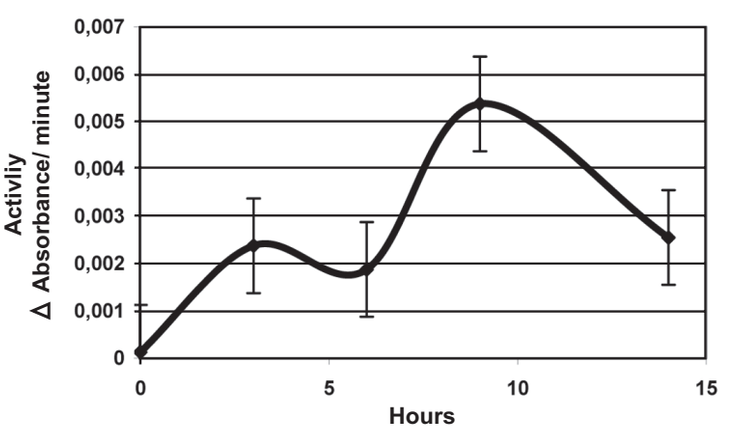

FIGURE 3 - Caspase 10-like activity (Δ absorbance / Δ minute) in *Lycopersicon hirsutum* over time following elicitation with 40 mM $CuCl_2$. The error bars corresponded to the standard deviation of two different time-independent assays.

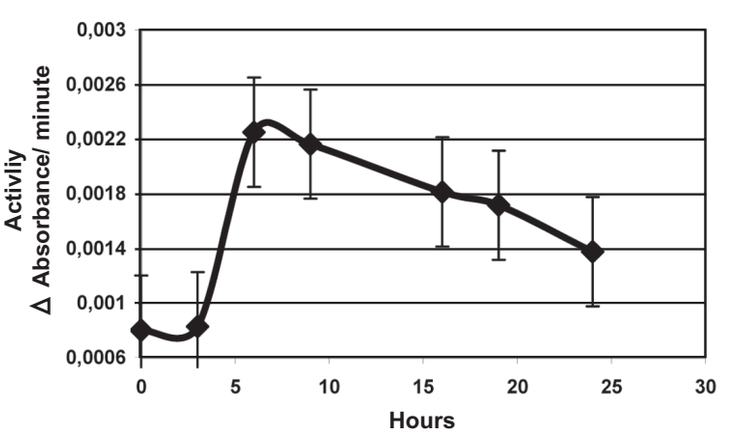

FIGURE 4 - Caspase 10-like activity (Δ absorbance / Δ minute) in *Lycopersicon esculentum* over time following elicitation with 40 mM $CuCl_2$ The error bars corresponded to the standard deviation of two different time-independent assays.

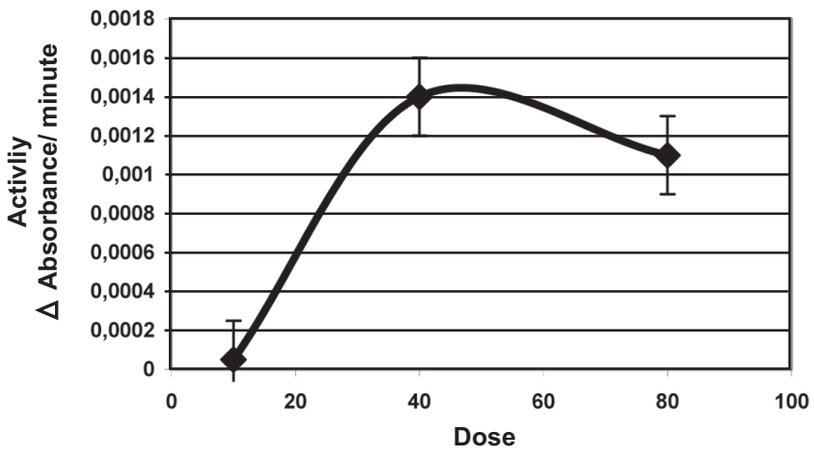

FIGURE 5 - Dose-response effect for caspase 10-like activity (Δ absorbance / Δ minute) in *Lycopersicon hirsutum* when elicited by 10, 40 or 80 mM $CuCl_2$. The error bars corresponded to the standard deviation of two different time-independent assays.

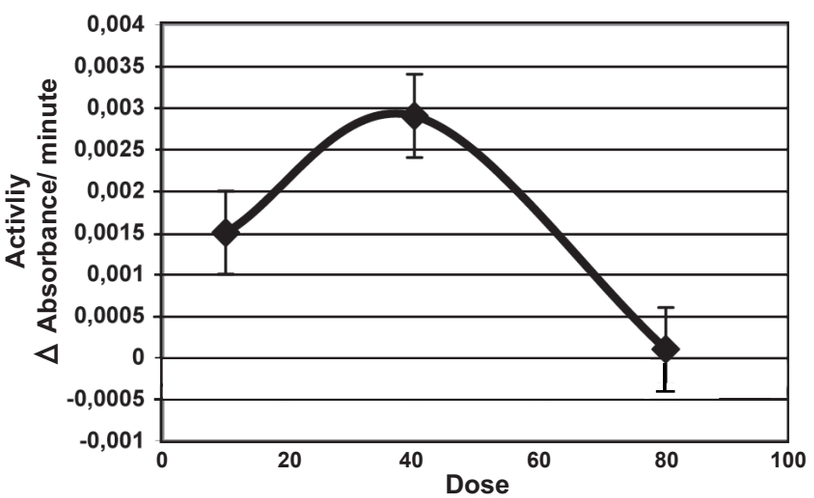

FIGURE 6 - Dose-response effect for caspase 10-like activity (Δ absorbance / Δ minute) in *Lycopersicon hirsutum* when elicited by 10, 40 or 80 mM $CuCl_2$. The error bars corresponded to the standard deviation of two different time-independent assays.

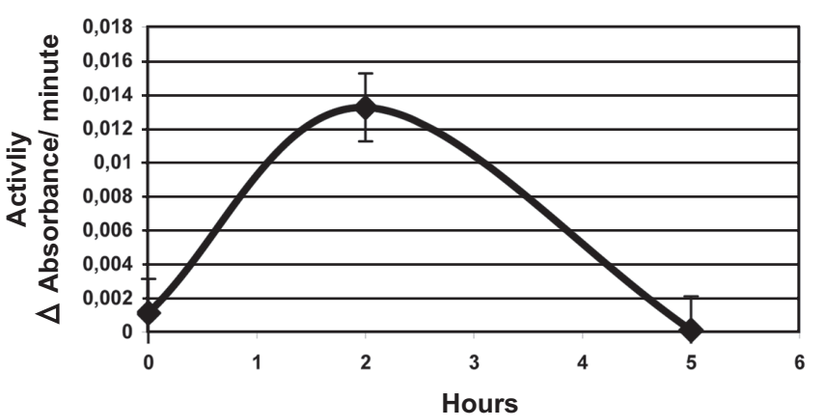

FIGURE 7 - Caspase 10-like activity (Δ absorbance / Δ minute) in *Lycopersicon hirsutum* over time following elicitation with 1% paraquat. The error bars corresponded to the standard deviation of two different time-independent assays.

tomato species. The level of caspase 10-like activity was higher for seedlings treated with 1% paraquat than for those treated with 3% paraquat treatment, and no activity was found in the 0.25% treatment (Figures 9-10).

Reproducibility of caspase 10-like activity assays under induction with copper and paraquat

The analysis did not show any statistically significant differences between the independent assays but did reveal differences in activity between the tomato species at 8.5 h after copper elicitation (Table 1). To confirm the reproducibility of the data, a Pearson correlation analysis was performed. The independent caspase activity assays for *L. hirsutum* had a high positive correlation of 0.86 with a Pr> value of 0.057; *L. esculentum* had a positive correlation of 0.65, which was not significant (Table 2). The reproducibility of the paraquat assays was similar to that for copper. No differences were observed between the assays (Table 1) or between the tomato species. The correlation coefficients revealed a higher significance, which corroborated the absence of differences between the assays, and also revealed a high degree of similarity in the nature of the phenomena (Table 2).

DISCUSSION

In the present work, we developed a protocol for the direct assessment of caspase 10-like activities in a *Lycopersicon* biological model, which lays the groundwork for future studies of plant reactions associated with apoptosis. *L. esculentum* and *L. hirsutum*, the tomato species studied, represent genotypes that are susceptible and resistant

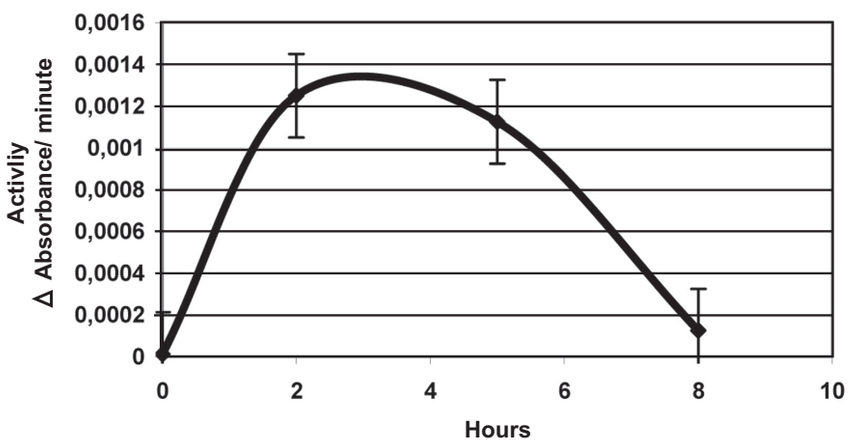

FIGURE 8 - Caspase 10-like activity (Δ absorbance / Δ minute) in *Lycopersicon esculentum* over time following elicitation with 1% paraquat. The error bars corresponded to the standard deviation of two different time-independent assays.

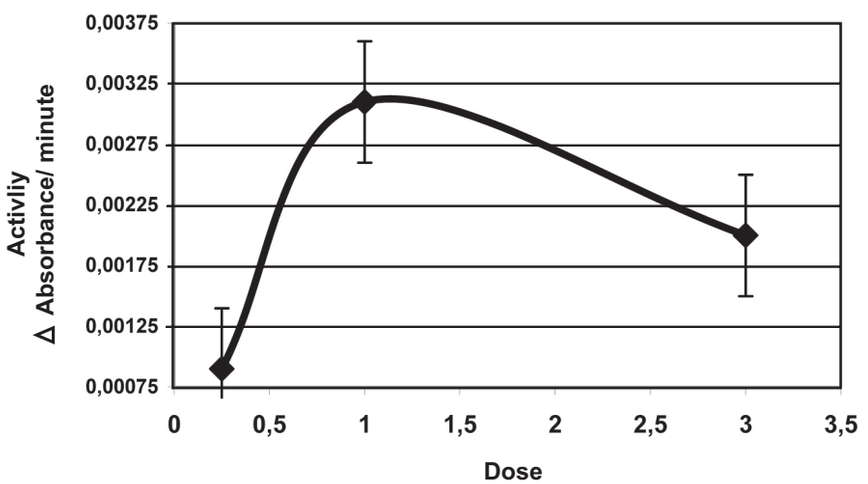

FIGURE 9 - Dose-response effect for caspase 10-like activity (Δ absorbance / Δ minute) in *Lycopersicon hirsutum* when elicited by 0.25, 1 and 3% paraquat. The error bars corresponded to the standard deviation of two different time-independent assays .

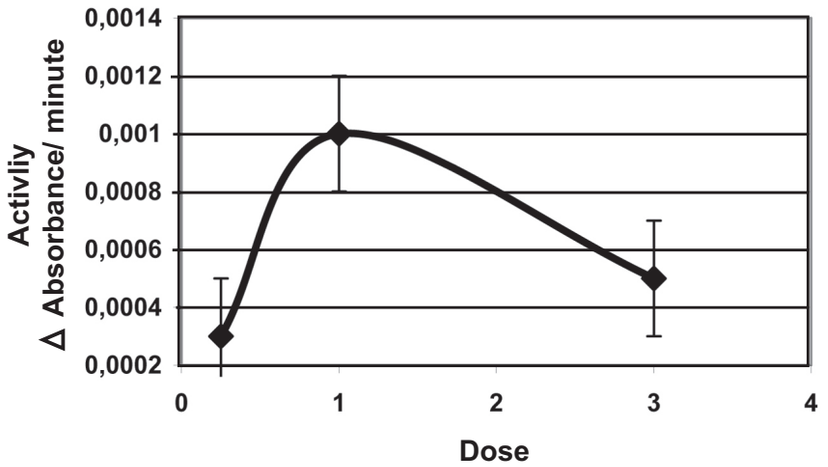

FIGURE 10 - Dose-response effect for caspase 10-like activity (Δ absorbance / Δ minute) in *Lycopersicon esculentum* when elicited by 0.25, 1 and 3% paraquat. The error bars corresponded to the standard deviation of two different time-independent assays .

TABLE 1 - Variance analysis and Tukey mean comparison test of the slope of the regressions, taken from the caspase 10-like activity kinetics, in two independent assays of *Lycopersicon hirsutum* and *Lycopersicon esculentum* elicited with 40 mM $CuCl_2$ and 1% paraquat

Treatment	Anova	Tukey test
Elicitation with copper	Pr > F=0.6494	a 0.0021700 assay 1 a 0.0018600 assay 2
Anova from 8.5 h	Pr > F= 0.0328	a 0.005450 *L. hirsutum* b 0.001788 *L. esculentum*
Elicitation with paraquat	Pr > F= 0.8840	a 0.002123 assay 1 a 0.001868 assay 2
Anova from 2.5 h	Pr > F= 0.0563	a 0.014375 *L. hirsutum* b 0.001563 *L. esculentum*

TABLE 2 - Pearson correlation analysis of the slope of the regressions, taken from the caspase 10-like activity kinetics, in two independent assays of *Lycopersicon hirsutum* and *Lycopersicon esculentum* elicited with 40 mM $CuCl_2$ and 1% paraquat

Assays	Correlation coefficient	Pr >F
Copper		
L. hirsutum: assay 1 vs assay 2	0.86653	0.0574
L. esculentum : assay 1 vs assay 2	0.65680	0.2285
Paraquat		
L. hirsutum: assay 1 vs assay 2	0.99012	0.0012
L. esculentum: assay 1 vs assay 2	0.96315	0.0084

to stress respectively, especially biotic stresses such as pathogen attacks by pathogens or insects. On the other hand, abiotic stress, which does not have a co-evolutive relation, can induce the expression of mechanisms closely linked to the immune response. Research during the past few years has elucidated cell death processes in plants, and included studies on caspases and caspase inhibitors (Del Pozo & Lam, 1998; Kusaka et al., 2004). Recent findings

corroborate the existence of highly conserved mechanisms in animals and plants. Measurements on cell suspensions of reactive oxygen species (ROS) are often used to assess cell death and effects due to caspase inhibitors. Although the mechanism of copper toxicity has not yet been described in detail, it is generally accepted that damage is generated following an oxidative stress route (Ke, 2007).

For the two tomato species in our tests, we propose a mechanism associated with apoptotic processes under elicitation with Cu^{2+}. In this model, we propose that copper induces specific caspase 10-like activities, which are associated with processes of apoptosis initiation. This type of induction probably would activate routes of cell death, which are considered to be exclusive of co-evolutive systems, similar to the plant-pathogen relationship. The direct determination of caspase 10-like activities on tomato seedlings using a specific substrate for mammals revealed the existence of highly conserved mechanisms in animals and plants. These mechanisms are related to apoptosis processes in tomatoes and are subsequent to abiotic induction. The caspase 10-like activity assays showed a maximum activity at 40 mM $CuCl_2$ and 8.5 h after the elicitation. The comparative observations for the two tomato species indicated that caspase 10-like activity is more stable in *L. hirsutum* and significantly higher than *L. esculentum.*

Observations in the elicitation experiments with paraquat suggest that plant interaction with a xenobiotic compound such as paraquat can activate processes in the genome, which are initially exclusive for plant-pathogen and plant-insect interactions. While paraquat inhibits the transit of electrons toward ferredoxin in photosystem I and destroys the cell with a consequent oxidative burst, a fully detailed mechanism of action has not been elucidated. A xenobiotic compound such as paraquat may be able to activate encoded processes in the genome. The finding of specific caspase 10-like activity in the two tomato species suggest a hitherto unreported mechanism for the action of paraquat.

Subsequent to a signaling process related to oxidative stress, the mechanisms associated with apoptosis and eventually with PCD processes would be activated. *L. hirsutum* differentially activated more processes that are linked to apoptosis and eventually to PCD. The elicitation with paraquat showed differences in the time reduction with regard to copper induction, suggesting that the acceleration of an apoptosis process was possibly associated with mechanisms related to higher production of ROS. The dose-response assays for both copper and paraquat suggest enzymatic processes that require adequate conditions for expressing the highest level of caspase 10-like activity.

ACKNOWLEDGMENTS

The authors acknowledge CORPOICA for providing the genetic resources. They also thank Grupo Interdisciplinario de Estudios Moleculares (GIEM) of Universidad de Antioquia for supporting this research and acknowledge Colciencias for support under its national Ph.D. program.

REFERENCES

Azcon-Bieto J, Talón M (2003) Fundamentos de fisiología vegetal. McGraw-Hill.

Baehrecke EH (2002) How death shapes life during development. Nature Reviews Molecular Cell Biology 3:779-787.

Boatright K, Salvesen G (2003) Mechanisms of caspase activation. Current Opinion in Cell Biology 15:725-731.

Burda K, Kruk J, Strzalka K, Schmid G (2002) Stimulation of oxygen evolution in photosystem II by copper II ions. Zeitschrift für Naturforschung 57:853-857.

Clarke PG, Clarke S (1995) Historic apoptosis. Nature 378:230.

Danon A, Delorme V, Mailhac N, Gallois P (2000) Plant programmed cell death: A common way to die. Plant Physiology and Biochemistry 38:647-655.

Del Pozo O, Lam E (1998) Caspase and programmed cell death in the hypersensitive response of plants to pathogens. Current Biology 8:1129-1132.

Earnshaw W, Martins L, Kaufmann S (1999) Mammalian caspases: Structure, activation, substrates and functions during apoptosis. Annual Review of Biochemistry 68:383-424.

Grayer R, Kokubun T (2001) Plant-fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants. Phytochemistry 56:253-263.

Godbole A, Varghese J, Sarin A, Mathew M (2003) VDAC is a conserved element of death pathways in plant and animal systems. Biochimica et Biophysica Acta 1642:87-96.

Golstein P, Aubry L, Levraud JP (2003) Cell-death alternative model organisms: why and which? Nature Reviews Molecular Cell Biology 4:798-807.

Hahn MG (1996) Microbial elicitors and their receptors in plants. Annual Review of Phytopathology 34:387-412.

Hammerschmidt R (1999) Phytoalexins: What have we learned after 60 years? Annual Review of Phytopathology 37:285-306.

Hengartner MO (2000) The biochemistry of apoptosis. Nature 407:770-776.

Hofius D, Tsitsigiannis D, Jones J, Mundy J (2007) Inducible cell death in plant immunity. Seminars in Cancer Biology 17:166-187.

Ke S. (2007) Effects of copper on the photosynthesis and oxidative metabolism of *Amaranthus tricolor* seedlings. Agricultural Sciences in China 6:1182-1192.

Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide ranging implications in tissue kinetics. British Journal of Cancer 26:239-257.

Kusaka K, Tada Y, Shigemi T, Sakamoto M, Nakayashiki H, Tosa Y, Mayama S (2004) Coordinate involvement of cysteine protease and nuclease in the executive phase of plant apoptosis. Federation of European Biochemical Societies Letters 578:363-367.

Mascher R, Fischer S, Scheiding W, Neagoe A, Bergmann H (2005) Exogenous 2-aminoethanol can diminish paraquat induced oxidative stress in barley (*Hordeum vulgare* L.). Plant Growth Regulation 45:103–112.

Mithofer A, Schulze B, Boland W (2004) Biotic and heavy metal stress response in plants: evidence for common signals. Federation of European Biochemical Societies Letters 566:1-5.

Vaux DL (1999) Caspases and apoptosis–biology and terminology. Cell Death and Differentiation 6:493-494.

Yakimova E, Kapchina-Toteva V, Woltering E (2007) Signal transduction events in aluminum-induced cell death in tomato suspension cells. Journal of Plant Physiology 164:702-708.

Yan N, Shi Y (2005) Mechanisms of apoptosis through structural biology. Annual Review of Cell and Developmental Biology 21:35-56.

Yoon JY, Shin JS, Shin DY, Hyun KH, Burgos N, Lee S, Yong IK (2011) Tolerance to paraquat-mediated oxidative and environmental stresses in squash (Cucurbita spp.) leaves of various ages. Pesticide Biochemistry and Physiology 99:65–76.

Yruela I, Puedo J, Alonso P, Picores R (1996) Photoinhibition of photosystem II from higher plants: effect of copper inhibition. Journal of Biology Chemistry 271:27408-15.

Zhaoa T, Davis L, Verpoortec R (2005) Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnology Advances 23:283-333.

TPP 159 - Received 11 August 2010 - Accepted 21 June 2011
Section Editor: John C. Sutton