

## CALCIUM ALLEVIATES STRESS IN PLANTS: INSIGHT INTO REGULATORY MECHANISMS

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### **Abstract**

*Plants as sessile organisms are constantly exposed to different stressors incidence: biotic and / or abiotic factors. Consequently, the viability of plants depends on their ability to perceive the changes that occur in their natural environment (either beneficial or harmful) and to appropriately respond physiologically, biochemically, and in terms of their development, to overcome and to counter the possible destructive effects. Plasma membrane, as the main selective barrier between the plant cell and the environment have different receptor proteins in its structure, to sense various external stimuli and transduce them to downstream intra- and intercellular signalling networks. An universal signal molecule is calcium and the calcium-sensing is of fundamental importance for extracellular calcium signalling and calcium homeostasis. Calcium is an important second messenger in signal transduction pathways, mediating various defence responses to the action under environmental stresses and in the regulation of many developmental processes in plants, being one of the most studied second messengers both in plant and animal fields. This brief review summarizes some results of recent research on the involvement of calcium in plant responses to stress factors action, addressing in particular scientific and practical importance of such knowledge, in order to improve plant tolerance to stress factors.*

**Keywords:** unfavorable factors, plant responses, messengers.

### **INTRODUCTION**

Plants are sessile and they must be able to sense their natural environment and undergo changes in their physiology and development in response to those environmental cues whether they are adverse or beneficial. Receptor proteins in the plasma membrane sense various environmental stimuli and transduce them to downstream intra- and intercellular signalling networks (Osakabe et al., 2013).

Calcium is a universal signalling molecule (Batistič and Kudla, 2012; Cheval et al., 2013; Moreno et al., 2014) and the calcium-sensing (CaS) receptor is of fundamental importance for extracellular calcium signalling and calcium homeostasis (reviewed by Bouschet et al., 2008). Calcium is an important second messenger in signal transduction pathways (Song et al., 2008; Achary et al., 2013), mediating various defence responses to the action under environmental stresses (Kim and Kim, 2006; Sano et al., 2006; Kader and Lindberg, 2010; Otulak and Garbaczewska, 2011; Waraich et al., 2011; Pan et al., 2012)

and in the regulation of many developmental processes in plants (Hepler, 2005; Hashimoto and Kudla, 2011; Kim et al., 2012), being one of the most studied second messenger both in plant and animal fields (Mazars et al., 2011). Several classes of calcium-sensing proteins, including calmodulin (CaM), calcineurin B-like (CBL) proteins, and calcium dependent protein kinases (CDPKs), have been identified in plants (Franz et al., 2011). Genome sequence analysis of *Arabidopsis* and rice has led to the identification of multigene families of these calcium signaling protein kinases (Das and Pandey, 2014). The Ca<sup>2+</sup>-signaling circuit consists of three major “nodes” - generation of a Ca<sup>2+</sup>-signature in response to a signal, recognition of the signature by Ca<sup>2+</sup> sensors and transduction of the signature message to targets that participate in producing signal-specific responses (Reddy and Reddy, 2004). Actually, Ca<sup>2+</sup> signatures encode information that specify downstream responses (Monshausen, 2012) and is now well established that the tight control of the temporal and spatial characteristics of cytosolic calcium alterations is considered to be responsible for

the specificity of various cellular responses, in particular to environment-induced stresses (Hong-Bo et al., 2008).

Plant  $\text{Ca}^{2+}$ -dependent protein kinases (CDPKs) are mono-molecular  $\text{Ca}^{2+}$ -sensor/protein kinase effector proteins, which perceive  $\text{Ca}^{2+}$  signals and translate them into protein phosphorylation and thus represent an ideal tool for signal transduction (Dubrovina et al., 2013; Jaworschi et al., 2012; Liese and Romeis, 2013). So, CDPKs proteins directly bind calcium ions before phosphorylating substrates involved in metabolism, osmosis, hormone response and stress signaling pathways (Valmonte et al., 2013).

Also, these are crucial calcium sensors involved in plant responses to pathogen infection (Fu et al., 2013). Calmodulin and calmodulin-like proteins are primary  $\text{Ca}^{2+}$  sensors that control diverse cellular functions by regulating the activity of various target proteins (Yang and Poovaiah, 2003; Perochon et al., 2011; Cheval et al., 2013; Robertson 2013).

In recent years, functions of calcium are subjected to many investigation and several excellent reviews have been published as regards as the importance of calcium in the different cellular compartments: cytosol, chloroplast, mitochondria (Manzoor et al., 2012), cellular organelles double membrane (Xiong et al., 2006,) nuclear calcium signaling (Mazars et al. 2011),  $\text{Ca}^{2+}$ -permeable channels in the plasma membrane of plant root cells (White, 2005),  $\text{Ca}^{2+}$  uptake into vacuoles (Pittman, 2011), plant defence responses to pathogens (Cheval et al., 2013; Thuleau et al., 2013) etc.

This brief review summarizes some results of recent research on the involvement of calcium in plant responses to stress factors action, addressing in particular scientific and practical importance of such knowledge, in order to improve plant tolerance to stress factors.

## **CALCIUM RECEIVES AND ALLEVIATES THE PLANT BIOTIC STRESS**

An increase in cellular calcium ion ( $\text{Ca}^{2+}$ ) concentration is acknowledged to be one of the earliest events occurring during the induction

of plant defence responses to a wide variety of pathogens (Vadassery and Oelmüller, 2009; Ma, 2011; Schulz et al., 2013).

Cyclic nucleotide gated channels (CNGCs) play important roles in facilitating  $\text{Ca}^{2+}$  uptake into plant cells and plant innate immunity.  $\text{Ca}^{2+}$  sensors, nitric oxide and other associate signaling components play pivotal roles in plant innate immune signaling cascade.  $\text{Ca}^{2+}$  homeostasis can be regulated by downstream signaling components (reviewd by Ma, 2011). Although it talks about the fact that calcium homeostasis is also provided by the participation of the Golgi complex, research conducted by Ordenes et al. (2012) have emphasized its non-involvement in the generation of cytosolic calcium, when applied different treatments. Moreover, Golgi apparatus express their dynamics modulated in part by the activity of  $\text{Ca}^{2+}$  pumps and hormones.

Liu et al. (2010) investigated calcium distribution and cell ultrastructural changes in wheat leaves invaded by leaf rust fungi. Electron-densed calcium precipitates were located mainly at intercellular spaces and in the vacuoles under resting conditions. After inoculation, extracellular calcium moved toward the cytoplasm across the cell wall and the plasma membrane in both resistant and susceptible plants. Also,  $\text{Ca}^{2+}$  and sphingoid long-chain bases (LCBs) may be interconnected to regulate cellular processes which lead either to plant susceptibility or to resistance mechanisms (Xiong et al., 2008; Thuleau et al., 2013). Nuclei respond to LCBs on their own independently of the cytosolic compartment (Xiong et al., 2008).

Otulak and Garbaczewska (2011) emphasized that infection of potato cv. Rywal with necrotic strains of *Potato virus Y* induced rapid hypersensitive response with highly localised increased accumulation of electron-dense calcium pyroantimonate deposits. The precipitates were founded along ER cisternae, chloroplast and mitochondria envelopes connected with PVY particles. Calcium deposits were detected in nucleus and in tracheary elements especially when virus particles were present inside.

Furthermore, cryptogin (a proteinaceous elicitor secreted by the oomycete *Phytophthora cryptogea*, which induces a hypersensitive

response in tobacco plants) (Amelot et al., 2012) and oligogalacturonides, two elicitors of plant defense reactions induced specific  $\text{Ca}^{2+}$  signatures in cytosol, mitochondria or chloroplasts. Also, depending on  $[\text{Ca}^{2+}]$  fluxes at the plasma membrane, cryptogein triggered a mitochondrial respiration increase and affected excess energy dissipation mechanisms in chloroplasts (Manzoor et al., 2012). After Amelot et al. (2012), results obtained on tobacco that the predominant functional gene categories affected by cryptogein included stress- and disease-related proteins, phenylpropanoid pathway, signaling components, transcription factors and cell wall reinforcement. The most  $\text{Ca}^{2+}$ -dependent transcripts upregulated by cryptogein were involved in defense responses or the oxylipin pathway.

Lachaud et al. (2010) emphasized the increase in tobacco BY-2 cells of the endogenous content of long chain bases by external application of d-erythro-sphinganine (DHS) and showed the nuclear calcium signature role in controlling d-erythro-sphinganine (DHS)-induced cell death in tobacco cells.

Studies by Vatsa et al. (2012) on the involvement of calcium in response to ergosterol (specific component of fungus membrane) using *Nicotiana plumbaginifolia* and *Nicotiana tabacum* cv *Xanthi* cells expressing apoaequorin in the cytosol have revealed the following: ergosterol caused a rapid, transient and biphasic free  $[\text{Ca}^{2+}]_{\text{cyt}}$  depending on its concentration; mobilizing calcium was specific for ergosterol; ergosterol induced changes in pH and  $[\text{Ca}^{2+}]_{\text{cyt}}$  changes were specifically desensitized after two subsequent applications of ergosterol; the extracellular alkalization and reactive oxygen species (ROS) production depended on calcium influx but, the ergosterol-induced mitogen-activated protein kinases (MAPK) activation was calcium-independent; ergosterol-induced ROS production is not linked to cell death and ergosterol does not induce any calcium elevation in the nucleus.

Kim et al. (2012) reported the first successful expression of a FRET (Förster Resonance Energy Transfer)-based  $\text{Ca}^{2+}$  biosensor in fungi. Time-lapse imaging of *Magnaporthe oryzae*, *Fusarium oxysporum*, and *Fusarium*

*graminearum* expressing this sensor showed that instead of a continuous gradient, the cytoplasmic  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_{\text{c}}$ ) change occurred in a pulsatile manner with no discernable gradient between pulses, and each species exhibited a distinct  $\text{Ca}^{2+}$  signature. Occurrence of pulsatile  $\text{Ca}^{2+}$  signatures was age and development dependent, and major  $[\text{Ca}^{2+}]_{\text{c}}$  transients were observed during hyphal branching, septum formation, differentiation into specialized plant infection structures, cell-cell contact and *in planta* growth. The data, materials and methods developed by Kim et al. (2012) will help understand the mechanism underpinning  $\text{Ca}^{2+}$ -mediated control of cellular and developmental changes, its role in polarized growth forms and the evolution of  $\text{Ca}^{2+}$  signaling across eukaryotic kingdoms.

For early detection and accurate determination of cytosolic ( $[\text{Ca}^{2+}]_{\text{cyt}}$ ) calcium variations, Verrillo et al. (2014) used the Cameleon YC 3.6 reporter protein expressed in *Arabidopsis thaliana* to quantify  $[\text{Ca}^{2+}]_{\text{cyt}}$  variations upon mechanical leaf damage herbivory by larvae of *Spodoptera littoralis* and *S. littoralis* oral secretions applied to mechanical damage. The authors reported for the first time the quantitative  $[\text{Ca}^{2+}]_{\text{cyt}}$  determination upon herbivory using the Cameleon calcium sensor.

Zebelo et al. (2012) have shown for the first time that the perception of volatile substances emitted by plants (eg tomatoes after the attack herbivores) and green leaf volatiles such as (*E*)-2-hexenal, (*Z*)-3-hexenal, (*Z*)-3-hexenyl acetate, the monoterpene  $\alpha$ -pinene, and the sesquiterpene  $\beta$ -caryophyllene is fast, second level, involving the alteration of the plasma membrane potential and the  $[\text{Ca}^{2+}]_{\text{cyt}}$  flux.

As regard as methyl jasmonate (MeJA) stimulation of defense responses in grapevine (*Vitis vinifera*), Faurie et al. (2009) investigated the involvement calcium to production of stilbenes, which are the grapevine phytoalexins. Its origin seemed to play a major role in MeJA-induced grapevine defense responses. Phytoalexin production was strongly affected if calcium from the influx plasma membrane was inhibited, whereas calcium from the intracellular compartments did not seem to be involved. By using as elicitors, cyclic oligosaccharides like cyclodextrins (CDs), alone or combined with methyl jasmonate

(MJ), Belchi-Navarro et al (2013) showed a very effective stimulating of the production of *trans*-resveratrol in *V. vinifera* suspension-cultured cells and a role of  $\text{Ca}^{2+}$  in mediating elicitor-induced *trans*-R was also emphasized.

Dubiella et al. (2012) identified calcium protein kinase 5 (CPK5) of the *Arabidopsis* calcium dependent protein kinase (CDPK) gene family as a positive regulator of innate immune signalling, with a dual function in rapid signal propagation and in the induction of prolonged transcriptional and phytohormone-mediated defense responses, mediating plant resistance to bacterial pathogens.

As well, the expression of *Oryza sativa* calcium protein kinase 10 (*OsCPK10*) was strongly induced following treatment with a *Magnaporthe grisea* elicitor. Overexpression of constitutively active *OsCPK10* in *Arabidopsis* enhanced the resistance to infection with *Pseudomonas syringae* pv. *tomato*, associated with elevated expression of both salicylic acid (SA)- and jasmonic acid (JA)-related defense genes. So, rice *OsCPK10* is a crucial regulator in plant immune responses, and it may regulate disease resistance by activating both SA- and JA-dependent defense responses (Fu et al., 2013).

## **CALCIUM RECEIVES AND ALLEVIATES THE PLANT ABIOTIC STRESS**

Abiotic stresses present major challenges in sustaining crop yield. They trigger responses involving molecular mechanisms for cellular adjustments, including signal perception and transduction cascades, transcriptional networks and adaptive metabolic pathways (reviewed by Chen et al, 2013).

Research conducted in tobacco revealed that both *oxidative and hipoosmotic stress* caused a growth of intracellular calcium content and induced cell cycle delay (Sano et al., 2006). Liu et al. (2010) showed that the increased cytosolic free  $\text{Ca}^{2+}$  by osmotic stress mainly comes from the extracellular and little is from the release of cytoplasmic calcium pool.

On the other hand, foliar application of  $\text{CaCl}_2$  enhanced adaptation of cucumber seedling to low light intensity and suboptimal temperature, as shown by the increase in net photosynthesis,

Rubisco activity, reduction in stress-induced malondialdehyde content, higher catalase activity and proline content (Liang et al., 2009). In tobacco plants exposed to heat stress, application of exogenous calcium chloride ( $\text{CaCl}_2$ ) (20 mM) improved the process of photosynthesis in relation to improving the stomatal conductance and improved the thermostability of oxygen-evolving complex, which might be due to less accumulation of reactive oxygen species (Tan et al., 2011).

The effect of calcium chloride on rice seedling growth under cadmium chloride ( $\text{CdCl}_2$ ) stress, as well as the possible role of endogenous nitric oxide (NO) in this process, was also studied (Zhang et al., 2012). The obtained results showed that Ca may alleviate *Cd toxicity* via endogenous NO with variation in the levels of nonprotein thiols (NPT), protein thiols (PBT), and matrix polysaccharides. As Cho et al (2012) noticed, Ca deficiency enhances Cd toxicity, and Ca may be required for heat-shock (HS) response in rice seedlings.

Some recent studies performed by Achary et al. (2013) for the first time, highlighted the the critical involvement of  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$  channels in the biphasic or hormetic mode of action of  $\text{Al}^{3+}$  leading to DNA damage at high doses and protection against genotoxic stress at low doses in *Alium cepa* root cells. Authors highlighted the importance  $\text{Ca}^{2+}$  homeostasis in maintaining the integrity of plant genome under  $\text{Al}^{3+}$ -induced oxidative stress and toxicity in plants.

Nasir Khan et al. (2012) studied the tolerance of excised mustard leaves to salt stress and showed that nitric oxide donor sodium nitroprusside (SNP) in association with  $\text{CaCl}_2$  plays a role in enhancing the tolerance of plants to salt stress by improving antioxidative defence system, osmolyte accumulation and ionic homeostasis.

The activation of the antioxidative system and secondary metabolites contents was also obtained by the addition of  $\text{CaCl}_2$  to  $\text{NaCl}$ -stressed *Penisetum* plants (Gobinathan et al., 2009). In barley seedlings studies, Guo et al. (2006) found that the alleviation of aluminum toxicity with Ca supplementation could be associated with less absorption of Al and the enhancement of the protective ability of the cell because of increased activity of the

antioxidative enzyme. For example, extracellular  $\text{Ca}^{2+}$  affects cell extension by modulating reactive oxygen species at the level of specific tissues and developmental stages. In this context of action, Afyanti and Chen (2014) noticed for the first time that sweet potato catalase activity is modulated by  $\text{CaCl}_2$  and sweet potato calmodulin, and plays an important role in  $\text{H}_2\text{O}_2$  homeostasis in mature leaves.

Regarding the involvement of calcium as a messenger in mediating signaling pathways in response to salt stress, Kim and Kim (2006) found the first known calcium-binding basic/helix-loop-helix-type transcription factor (*AtNIG1*), involved in plant salt stress signaling. Also, recently, Xi et al. (2012) indicated that *AtGT2L* (GT factors are a family of plant-specific transcription factors with conserved trihelix DNA-binding domains that bind GT elements) is a  $\text{Ca}^{2+}$ /CaM-binding nuclear transcription factor involved in plant responses to cold and salt stresses. Hua et al. (2008) mentioned that the effects of salt-stress on plants involve not only the water stress caused by low osmotic pressure, but also the toxicity of excess  $\text{Na}^+$ . A large amount of  $\text{Na}^+$  entering cells would reduce  $\text{K}^+$  uptake, which leads to an imbalance of K:Na ratio in cells. NaCl-induced changes of membrane potential of root epidermal cells of maize (*Zea mays* L., Denghai 11) seedlings and the depolarization became greater and faster with increasing of NaCl concentration. The addition of calcium postponed the depolarization, decreased the degree of depolarization and had a positive influence in the integrality of cell membrane.

As a practical matter, experiments performed in tomato (*Lycopersicon esculentum* Mill.) cv. "Target F1" (Tuna et al., 2007) showed that supplementary calcium sulphate application in nutrient solutions at high NaCl concentrations significantly improved growth and physiological variables affected by salt stress (e.g. plant growth, fruit yield, and membrane permeability) and also increased leaf  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and N in tomato plants. Also, reducing concentration of  $\text{Na}^+$  (because of cation competition in root zone) in leaves could offer an economical and simple solution to tomato crop production problems caused by high salinity. Application of boron (B) (up to 55.8

$\mu\text{mol/L}$ ) and  $\text{Ca}^{2+}$  (up to 2.72 mmol/L) increased bacterial population of *Pisum sativum* L. cv. Argona inoculated with *Rhizobium leguminosarum* bv. *viciae* 3841 in salt-stressed nodules. Salt stress produced cells with walls dramatically altered or even degraded in several zones, and pectin polysaccharides, increased in cells under salinity. These effects resembled typical effects of B-deficiency reactions in cell walls, and the increase of both  $\text{Ca}^{2+}$  and especially B also prevented these alterations (Bolaños et al., 2003). Studies carried out by Supanjani et al. (2006) in soybean (*Glycine max* [L.] Merr.), by inoculation with rhizobia or application of Nod factors (lipo-chitooligosaccharides) suggested that in addition to its known role in provision of nitrogen fixation, rhizobia also improves early calcium uptake into soybean plants.

Nowadays, substantial experimental evidence points to a pivotal role for calcium-dependent protein kinases (CDPKs).

Quantitative real time PCR (qRT-PCR) analysis in the transgenic *Arabidopsis* identified several members of CDPKs as regulators for plants response to abscisic acid (ABA) signaling (Jiang et al., 2013). Overexpression of *ZmCPK4*, from maize in the transgenic *Arabidopsis* enhanced ABA sensitivity in seed germination, seedling growth and stomatal movement. The transgenic plants also enhanced drought stress tolerance. So, the results suggest that *ZmCPK4* might be involved in ABA-mediated regulation of stomatal closure in response to drought stress. Chen et al. (2011) isolated CDPK gene, *NtCDPK12* from common tobacco (*Nicotiana tabacum*) leaves and showed that this gene was highly expressed in stems and increased in roots treated with high-salt or subjected to drought stress. Also, a CDPK gene cloned from *Populus euphratica*, designated as *PeCPK10* (localized within the nucleus and cytosol), was rapidly induced by salt, cold, and drought stresses (Chen et al., 2013) and a positive regulator responsive to cold and drought stresses in *P. euphratica* was proposed. Dubrovina et al. (2013) studied expression of CDPK genes under osmotic and temperature stress treatments in wild-growing grapevine *Vitis amurensis* Rupr. Using RT-PCR technique they identified 13 CDPK genes that

are actively expressed in healthy *V. amurensis* cuttings under high salt, high mannitol, desiccation, and temperature stress conditions. 12 CDPKs, namely *VaCPK1*, *VaCPK2*, *VaCPK3*, *VaCPK9*, *VaCPK13*, *VaCPK16*, *VaCPK20*, *VaCPK21*, *VaCPK25*, *VaCPK26*, *VaCPK29* and *VaCPK30*, were novel for Vitaceae, and their full cDNAs were obtained and described. The variability in their organ-specific expression patterns indicates that the enzymes perform distinct biological functions. Rao et al (2010) have characterized the function of a calcium sensor-interacting protein kinase, *OsCIPK03*, in the salt stress in the response of rice (*Oryza sativa* L.) and suggested that it plays a negative regulator of rice tolerance to salt stress. On the other hand, Asano et al. (2012) suggest that *OsCPK12* promotes tolerance to salt stress by reducing the accumulation of ROS and *OsCPK12-OX* seedlings had increased sensitivity to abscisic acid (ABA) and increased susceptibility to blast fungus, probably resulting from the repression of ROS production and/or the involvement of *OsCPK12* in the ABA signaling pathway. Malabadi and Staden (2006) showed that embryogenic cells require minimal concentrations of  $\text{Ca}^{2+}$  during pretreatment for the expression of the cold-enhancement of embryogenesis in mature *Pinus patula* tissues. Involvement of calcium in enhancing plant tolerance to the action of metals such as aluminum was noticed recently by Maejima et al. (2014). The authors explained this by the influence of the phosphate group of phospholipid composition of the plasmalemma and carboxyl groups of pectins present in cell walls, which are usually neutralized by calcium (Ca). It is known that in many plants of the family Leguminosae accumulate calcium oxalate (CaOx) crystals, whose distribution and accumulation depends on the climate change. Research conducted by Brown et al. (2013) in phyllodes of the leguminous *Acacia* sect. *Juliflorae* (Benth.) C. Moore & Betche from four climate zones along aridity gradient year suggested that both aridity and soil calcium levels have an important role in the precipitation of CaOx in *Acacia* most likely function in bulk calcium regulation.

The calcium concentration and control it also presents a great practical importance for plant growth on artificial mineral nutrient solutions. Given the need for ion selective sensors in greenhouses and protected areas in general to allow nutrients and water consumption reduction, while improving production, Bamsey and al (2014) have developed ion-selective bulk optodes tailored specifically for calcium monitoring in hydroponic nutrient solution. Studies have focused on theoretical and experimental production of calcium-selective membranes based upon the calcium ionophore ETH 1001 and hydrogen ion chromoionophore ETH 5350 incorporated into highly plasticized PVC membranes. The developed membranes have been tested within a portable fiber optic instrument that could be directly incorporated into an operational plant growth system to selectively measure calcium. Experiments carried out by Wu et al. (2012) have revealed that calcium applied in nutrient solution used for maize (*Zea mays* L.) subjected to variable water conditions influenced the hydraulic properties of the whole roots ( $Lp_r$ ) and cortical cells ( $Lp_{\text{cell}}$ ). Under well-watered conditions, extra  $\text{Ca}^{2+}$  significantly increased the root Ca content, total root length, and lateral root number; however, it reduced the root cortical cell volume,  $Lp_r$ , and  $Lp_{\text{cell}}$ .  $\text{Hg}^{2+}$  inhibition experiments suggested that extra  $\text{Ca}^{2+}$  could reduce the contribution of the cell-to-cell water flow pathway. Osmotic stress (10% PEG6000) significantly decreased the cortical cell volume,  $Lp_r$ , and  $Lp_{\text{cell}}$  in the control plants, but smaller decreases were observed in the extra  $\text{Ca}^{2+}$  plants. Extra  $\text{Ca}^{2+}$  may adjust the contribution of cell-to-cell pathway by regulating the expression and/or activity levels of AQPs according to water availability; this regulation may weaken negative effects and optimize water use. Some of the ameliorative mechanisms of  $\text{Ca}^{2+}$  on salt stress at the cellular and tissue levels of *Zea mays* L. plants grown in nutrient solution containing 1 or 80 mM NaCl with various  $\text{Ca}^{2+}$  levels were also showed by Shores et al. (2010). Plant species differ markedly in terms of absorption and retention of calcium, especially in terms of absorbed calcium sequestration capacity. Of the six species studied by Borer et

al. (2012), four were found to sequester 40-90% of foliar calcium in chemically-unavailable form, and one of these obviously limited calcium uptake. Such researches offer new insights into the physiology of foliar Ca tolerance in plants growing in high-Ca conditions. Studies performed by Chen et al. (2013) on grapevine cuttings in a hydroponic system exposed to Cu-spiked solutions, with two Ca backgrounds (0.5 and 5 mM) for 15 days revealed that increase of Ca background concentrations would enhance Cu to be accumulated by root, but not translocated into the leaf.

## CONCLUSIONS

Plant response to biotic and abiotic stressors is one of great complexity, a wide variability and mutual interrelation is expressed through multiple and highly varied manifestations at molecular, cellular, tissue, organ level and plant as a whole. It is unlikely that in the near future this issue will be resolved and by right to receive answers expected by scientists, agronomists (as producers) and consumers.

However, progress regarding the involvement of calcium as a second messenger in enhancing plant tolerance to the action of stress factors, offers hope that further research will provide insight into successful, to have a clearer picture about the molecular, physiological and biochemical mechanisms of plant response and adaptation to unfavorable conditions of life.

As mentioned Zagorchev et al. (2014), charging different signals caused by stressors and subsequent operation of various signaling pathways should be the topics of particular interest in the future research.

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