QUADRUPLE IMMUNE RESPONSE OF PLANTS TO PATHOGENS AFTER PRETREATMENT WITH DIFFERENT DOSES OF TRACE ELEMENTS

Ernő Tyihák[a]*, Ágnes M. Móricz[a], Mihály Szilágyi[b], Ferenc Billes[c]

Paper was presented at the 4th International Symposium on Trace Elements on the Food Chain, Friends or Foes, 15-17 November, 2012, Visegrád, Hungary

Keywords: formaldehyde, hormesis, inducer, pretreatment, quadruple immune response, trace elements.

The enzymatic and/or non-enzymatic methylation / hydroxymethylation of main trace elements renders a special, indispensable bridge between biological (organic) and inorganic world. These modified trace elements are potential formaldehyde (HCHO) generators and HCHO formed from them can participate in different characteristic interactions. On the basis of up-to-date biochemical results with HCHO it is supposed that trace elements as HCHO carriers transport HCHO molecules in dose-dependent level to different points of a given biological unit. On the basis of experiences with the time- and dose-dependent double immune response of plants to pathogens, a logical step was to extend it to the total Avogadro number range (in vivo conditions) in the case of trace elements as potential inducers as well. These new findings support that HCHO and its reaction products (mainly O_3) as drastic molecules are responsible for the immuno-stimulating activity of trace elements as inducers. It is especially important that there are always four bioequivalent immunostimulating activity ranges in plants for the pretreatment with different doses of trace elements similar to organic compounds. It has to be noted that the trace elements as inducers actually don't participate directly in the induction of the immunostimulating effect similar to organic inducers.

* Corresponding Authors
Fax: 00-36-1-48-77-555
E-Mail: tyihak.erno@agrar.mta.hu
[a] Centre for Agricultural Research, Plant Protection Institute, Hungarian Academy of Sciences, H-1525 Budapest, POB 102, Hungary
[b] Research Institute for Animal Breeding and Nutrition, H-2053 Herceghalom, Hungary
[c] Department of Physical Chemistry, Budapest University of Technology and Economics, H-1521 Budapest, Budafoki út 8, Hungary

Introduction

The recent increase in the number of studies concerning the defence mechanisms of plants reflects the interest in a better understanding of plant immunity as pre-formed (constitutive, innate) defence systems and adaptive immunity as induced resistance mechanisms. [1-4]

Plant immunity is composed of several layers including robust pre- and post-invasion defences[5]. It is fact that plants successfully use pre-formed physical and chemical innate defence systems as well as inducible adaptive immune strategies to pathogens, although, circulating cells, immunglobulin molecules and phagocytic processes lack in plant tissues [6].

Until recently, the mechanisms of antibody-mediated immunity in humans and animals against microorganisms are not well known and it is fact that until now immunoglobulin-like proteins have not been found in plants. Therefore, it is high time to find a common point (e.g. biochemical pathway) that is valid for innate and adaptive immunity in animals and plants similar. Recent studies open new horizons in this field: antibodies can catalyze the generation of very reactive biological oxidants including ozone (O_3) – through H_2O_3 -from the interaction of singlet oxygen ($^1O_2$) with H_2O.[7-9] It is obvious that the formation of $^1O_2$ is crucial step in this complicated system. Both HCHO and H_2O_2 can be formed continuously and are present (HCHO mainly in hydroxymethyl groups) intracellular and extra-cellular by almost all cells[10,11]. These two reactive molecules can interact (also endogenously) and the very reactive $^1O_2$ and excited HCHO can be formed[10,12], that is, the formation of $^1O_2$ may be continuous in all cells at different levels. According to preliminary experiments these very reactive molecules (from HCHO to O_3) can be formed in plant tissues and biological world in general.[13,14]

Resistance phase of stress syndrome can be activated by biotic (e.g. avirulent forms of pathogens, incompatible races of pathogens)[15-18] and by abiotic (inorganic and organic compounds, UV-ray, high temperature, etc.) inducers.[19-22] Induced resistance means an improvement of the natural (innate) resistance of plants without alteration of their genome. Induced resistance is generally systemic, because the defensive capacity is increased not only in the primary infected plant tissues, but also in distant parts of the plant.

Among the abiotic inducers the trace elements with double effect[23] and as HCHO carriers [24] play a determining role. The aim of this work is to demonstrate the basic elements of the time- and dose-dependent quadruple, non-linear, however, specific immune response of plants to pathogens regarding interactions and function of formaldehyde (HCHO) and its reaction products in it using different doses of trace elements for pretreatment of plants.

Experimental

Chemicals

All chemicals were obtained from Merck Hungary (Budapest, Hungary) and Sigma Co (St Louis, USA) as well as Reanal Chemical Co (Budapest, Hungary).

Plant and pathogens

Bean (*Phaseolus vulgaris* cv. Debreceni tarka, Békési fehér) plants were cultivated in commercial compost in the greenhouse held at 24°C with 4 hours supplementary light (6000 lux) in the morning and evening to give a light period of at least 16 hours per day. The investigations were limited to primary leaves. Bean rust (*Uromyces phaseoli*) was maintained on bean in the greenhouse.

Biochemical immunization (pretreatment) and inoculation

Aqueous solutions of potential inducers (decimal dilution) were used. The solutions were sprayed onto the abaxial leaf surface in case of bean plants. Plants treated with water were used as controls.

Bean plants were inoculated with an aqueous spore suspension of bean rust 4 days after pre-treatment with inducer and then incubated at 20-22°C for 24 hours at high relative humidity.

Capture of HCHO in bean plants (in vivo) with dimedone

To aqueous solution of inducers dimedone (a HCHO capturing molecule) was given for the elimination of this reactive molecule. The decimal dilution was carried out similar to biochemical immunization process for all inducers and capturer.

Evaluation of data

Pustule densities were assessed 9-10 days after inoculation and disease severity (infection rate, infectivity) was expressed as the number of pustules cm^{-2} (by means of a homemade pattern) in the case of bean plants. The mathematical evaluation of the data was carried out by moving average calculations using an appropriate software.

Results and Discussion

On the basis of earlier experiences with the time-and dose-dependent double immune response of plants to pathogens[13] a logical step was to extend it to the total Avogadro number range for example from 10^{-1} to 10^{-23} mol/L. Figure 1 supports the original conception. As it can be seen at the 4 day interval between pre-treatment and inoculation in bean-*Uromyces* sp. relationship there is a characteristic time- and dose-dependent quadruple immune response of plants to pathogens using in this case N-methyl-L-methionine as special natural amino acid derivative (organic compound) with N-and S-methyl groups as potential formaldehyde generators .[25]

It seems that Cu(II) might be an especially important and suitable agent (inducer) for such a pretreatment of plants because this molecule does not contain methyl group (HCHO precursor) but it can mobilize and transport a relatively high amount of HCHO (four molecules) in hydroxymethyl groups from plant tissues and/or pathogen cells according to recent observations[24] and there are preliminary immunization results with this trace element.[23]

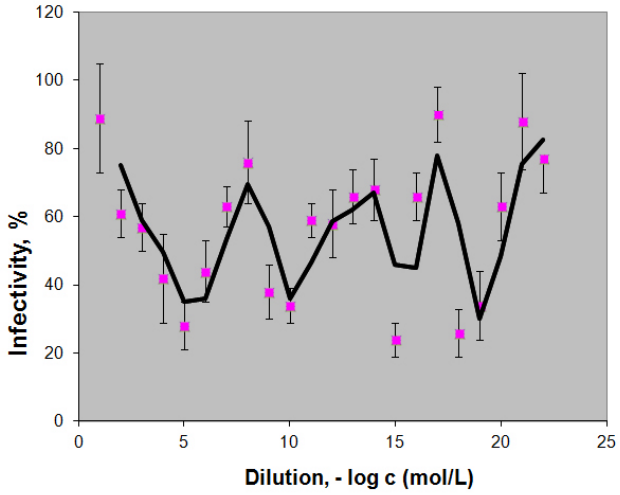

Figure 1. Quadruple immune response of bean plants to *Uromyces phaseoli* using N-methyl-L-methionine as inducer for pretreatment (with permission, from Ref. [25]).

It is obvious that there is a non-linearity for the Avogadro number range and it follows from these results that there is a molecular order in the induced resistance of plants (e.g. there is a similar or same inactive range between two active ranges and there is receptor-directed exact dose-effect etc.).The Figure 1 illustrates clearly that methyl-methionine generated 4 immunostimulating ranges with practically same activity intensity, that is, for example 10^{-5} mol/L (at allopathic dose-range) and 10^{-20} or 10^{-19} mol/L (homeopathic dose-range yet within Avogadro range) generate practically same immunostimulating activity.[25] These results with a non-toxic, endogenous molecule show – among others – that in the immunostimulating activity the given inducer doesn't participate directly in the inducing effect. These results with different doses of some trace elements support also the preliminary results with HCHO reactions of trace elements [26] including the quantumchemical calculations .[27] It has to be noted down now to this figure that the activity of the last, very low dose will be especially interesting for understanding and answering the unique phenomena after Avogadro range.

Our present results illustrate clearly that the trace elements can also generate hormesis effect (Figure 2 and 3) as the organic compounds in which HCHO and its special reaction products (e.g. O_3) play a crucial role (Figure 4). These results with trace elements eliminate also the idea on the two-phase hormesis.[28,29] Our results show that hormesis and its phases are in the resistance phase. On the basis of quadruple immune response of plants to pathogens the resistance phase of stress syndrome can be divided into four equivalent (?) parts. The further study of trace elements in vitro (BioArena[14]) and in vivo (e.g. greenhouse[25]) conditions regarding to HCHO/O_3 idea assures further revelation.

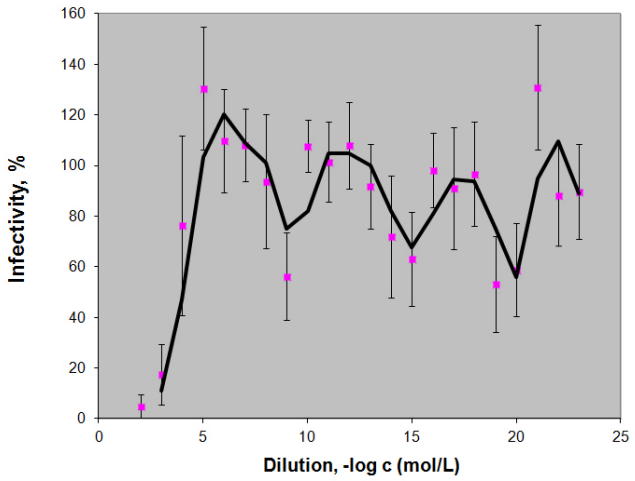

Figure 2. Effect of Cu(II) ion pretreatment on the resistance potential of bean plants to *Uromyces phaseoli*

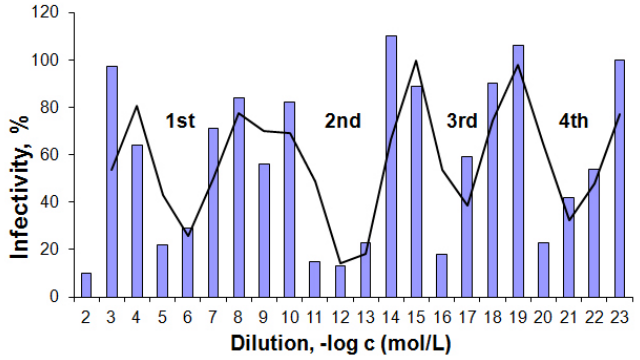

Figure 3. Effect of Ni(II) ion on the resistance potential of bean plants to *Uromyces phaseoli*

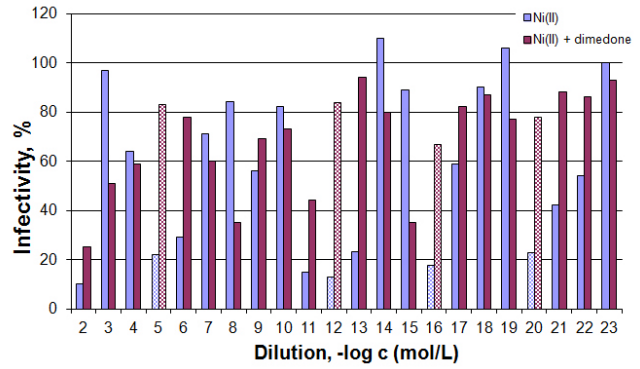

Figure 4. Effect of dimedone on the immunstimulating activity of Ni (II) ion in bean-*Uromyces phaseoli* relationship. The checked columns mean the four active ranges and their elimination with dimedone as a HCHO capturer.

References

[1] Cohn, J., Sessa, G., Martin, G. B., *Curr. Opin. Immunol*., **2001**,*13*, 55.

[2] Bostock,R. M., *Annu. Rev. Phytopathol*., **2005**, *43*, 545.

[3] Zhai, Z., Liu ,Y., Wu, L., Senchina, D.S., Wurtele, E. S., Murphy., P. A., Kohut, M. L., Cunnick, J. E., *J. Med. Food*, **2007**, *10*, 423.

[4] Jung, H. W., Tschaplinski, T .J., Wang, L., Glazebrook, J., Greenberg, J.T., *Science*, **2009**,*324*, , 89.

[5] Da Cunha, L., McFall, A. J., Mackey, D., *Microbes Infect*., **2006**, *8*, 1372.

[6] Menezes, H., Jased, C., *Comp. Biochem. Phys. C.*, **2002**, *132***,** 1.

[7] Wentworth, A. D., Jones, L. H., Wentworth, P. Jr., Janda, K. D., Lerner, R. A., *P. Natl. Acad. Sci. USA.*, **2000,** *97*, 10930.

[8] Wentworth, P., Jr., Janda, K. D., *Cell Biochem. Biophys*., **2001**, *35*, 63.

[9] Babior, B. M., Takeuchi, C., Ruedi, J., Gutierrez, A., Wentworth, P, Jr., *P. Natl. Acad. Sci. USA*., **2003**, *100*, 3031.

[10] Tyihák, E., Rozsnyay, S., Sárdi, E., Gullner, G., Trézl, L., Gáborjányi. R., *Acta Biol. Acad..Sci. H*., **1994**, *45*, 3.

[11] Tyihák, E., Trézl, L., Szende, B., *Ann. N. Y. Acad. Sci*., **1998**, *851*, 259.

[12] Trézl, L., Pipek, J., *J. Mol. Struc. – Theochem*., **1988**, *170*, 213.

[13] Tyihák, E., In: Teixeira da Silva, J.A., (editor), *Floriculture, Ornamental and Plant Biotechnology, Advances and Topical Issues*. Vol. III, London (UK), Global Science Books, **2006**, 380.

[14] Tyihák, E., Móricz, Á. M., Ott, P. G., *J. Planar Chromat.*, **2008**, *21*, 77.

[15] Hostetter, M. K., *Curr. Opin. Microbiol*., **2000,** 3, 344.

[16] Klement, Z., Bozsó, Z., Kecskés, M. L., Besenyei, E., Celleng, A., Ott, P. G., *Pest Manag. Sci*., **2003**, *59*, 465.

[17] Kothari, I. L., Patel, M., *Indian J. Exp. Biol*., **2004**, *42***,** 244.

[18] Xia, Y., Gao, O. M., Yu, K., Lapchyk, L., Navarre, D., Hildebrand, D., Kachroo, A., Kachroo, P., *Cell Host Microbe*. **2009**, *5*, 151.

[19] Alvarez, M. E., Pennell, R. I., Meijer, P. J., Ishikawa, A., Dixon, R. A., Lamb, C., *Cell*, **1998**, *192*, *773*.

[20] Zhao, H., Wang, B-Ch., Zhao, H.-Ch., Wang, J. B., *Colloid Surface B*., **2005**, *44*, 36.

[21] Park, S. W., Kaimovo, E., Kumar, D., Mosher, S., Klessig, D. F., *Science*, **2007**, *318*, 31.

[22] Endo, J-i, Takahashi, W., Ikegami, T., Beppu, T., Tanaka, O., *Biochem.*, ***2009****; 73*, 183.

[23] Tyihák, E., Móricz, Á. M., Ott, P. G., Király-Véghely, Zs., Kátay, Gy., In: *Procs Intern. Symp. On Trace Elements in the Food Chain*, Budapest, May 25-27, (Eds.: Szilágyi, M., Szentmihályi, K.), **2006**, 394.

[24] Tyihák, E., Takátsy, A., Móricz, Á. M., Ott, P. G., Ohmacht, R., In: *Trace Elements in the Food Chain, Vol.3. Deficiency or Excess of Trace Elements in the Environment as a Risk of Health*. Budapest, Hungary, (Eds.: Szilágyi, M., Szentmihályi, K.), **2009,** 392.

[25] Tyihák, E., Mincsovics, E., *J. Planar Chromat*., **2010**, *23*, 382.

[26] Móricz, Á. M., Ott, P. G., Szilágyi, M., Otta, K., Tyihák, E., *Acta Biol. Acad. Sci. H*., **2007**, *58*, 301.

[27] Billes, F., Mohammed-Ziegler, I., Mikosch, H., Tyihák, E., *Spectrochim. Acta A*., **2007**, *68*, 669.

[28] Calabrese, E. J., *Mutat. Res*., **2002**, *511*, 181.

[29] Calabrese, E. J., Mattson, M.P., Calabrese, V., *Human. Exp. Toxicol*., **2010**, *29*, 1034.

Received: 18.10.2012.
Accepted: 14.11.2012.