ORIGINAL PAPER

EFFECT OF ZINC SUPPLEMETATION ON GROWTH, BIOCHEMICAL PROCESS AND YIELD IN ZEA MAYS

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Abstract. *This paper aims the effect of zinc at different concentrations on development of Zea mays and the characteristic reactions generated or inhibited by the zinc. The plants were raised in well-established conditions, and Zn was administered at variable levels ranging from acute deficiency (sample 0, witness) to excess (100 mg/kg, 300 mg/kg and 700 mg/kg). Zinc is an essential micronutrient required by plants for normal growth and development. This metal was determined by Flame Atomic Absorption Spectrometry (FAAS). At lower amount or even absence of zinc, symptoms of Zn deficiency were depression in growth, chlorosis and necrosis of mature leaves, along with development of purple pigmentation at leaf margins. Zinc at less and more than 100 mg/kg reduced vegetative yield, seed weight, concentration of chlorophyll (a and b), sugars, and protein and elevated the concentration of phenols and activity of different enzymes in metabolism processes. In addition to various changes in metabolism of Zea mays, Zn at higher levels induced symptoms of toxicity on leaves as necrosis, and reduction in growth and leaf size. Phosphorus content in leaves decreased concomitantly with an increase in Zn supply from 100 mg/kg to 700 mg/kg. The toxicity of higher amount of zinc added of plant is expected to be in the last samples Zn3 as well when the yield of growth is less according with the data from literature.*

Keywords: *Zea mays, zinc, FAAS, enzyme, biochemical process.*

1. INTRODUCTION

The cereals, known as *Geamineae*, are the most important staple crops from the world. Cereal grains are grown in greater quantities (even in Antarctica) and provide more food energy worldwide than any other type of crop [1].

The maize (*Zea mays*) is a basic cereal plant. This plant takes the first place in the world (818823434 tones in 2009) (Food and Agriculture Organization of the United Nations) to the global cereal crops. In present, its study is very important to could avoid the future problems that could trigger a global food crisis. If the *Geamineae* plants would disappear one

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of the old enemy of wheat *Puccinia graminis*, a fungus known as black rust could destroy 90% of global wheat crops. This fungus classified as Ug99, could leave the planet to starve, if that would spread from the contaminated zones as Kenya, Ethiopia, Sudan, Yemen around the world. It is very well known that the great danger is the environmental pollution.

Along with the higher levels of pollution, plants become weaker in the face of all kinds of threat. Acid rains, heavy metals and agricultural plant selection by the productivity criteria can lead to loss of other important qualities of plants. In plants occurs the acceleration of undesired processes, inhibition of important reactions, formation of toxic products (for plants, but also for the consumer). Heavy metals are an important worldwide oldest problem against Ug99 and perhaps less evident to the effect. These two combined factors could lead to fatal consequences. Cereals grown in developed countries near industrial objectives will be more vulnerable to this fungus.

The zinc is a heavy metal and a biogenic element as well. His role for plants was shown for the first time in 1872 by Kliment Timiryazeva [2] when he observed that zinc can remove the chlorosis symptoms to the plants. This metal supports the normal activity of over 300 enzymes such as anhydrases, dehydrogenases, proteinases, peptidases [3]. The principal functions of zinc are [4]: the growth hormone production (auxins); the seed formation; accelerates the maturation of plants; increase in height of plants; protein synthesis; stabilization of some protein structure; it is necessary to divide the cells; participate in the nitrogen cycle plants; transformation and carbohydrate consumption. In many studies [2] was shown that the lower concentration of zinc can lead to the decrease of chlorophyll amount from leaves. At maize the colour of leaves are change, that main appear certain spots which are becoming lighter and are parallel with the stems of leaves.

This paper aims the effect of zinc at different concentrations on development of *Zea mays* and the characteristic reactions generated or inhibited by the zinc.

2. EXPERIMENTAL

The biochemical analyses are performed by using the *Zea mays* seeds germinated for 7 days in advance, in deionized water. The germinated seeds were planted in soils administrated with zinc at different concentrations as, 100 mg/kg, 300 mg/kg and 700 mg/kg. As standard, it was used an uncontaminated soil. The evolution of plants was analyzed in 13 days. The height of *Zea mays* was measured at the same hour. In this paper it aims the study of negative effects of zinc in extreme conditions even though this metal is a microelement. In order to not involve the action of other factors, plants were grown under laboratory conditions and watered with distilled water, which not contained heavy metals for not influence the plant growth.

The concentrations of Zn in *Zea mays* collected leaves were performed by Flame Atomic Absorption Spectrometry (FAAS) using an Avanta GBC spectrometer. Dried leave samples were digested in an acid solution using a Berghof MWS-2 microwave digestion system. Dried samples (300 mg) were introduced into the digestion vessels; then 8 mL nitric acid and 10 mL hydrogen peroxide were added. Certified Standard Reference Material SRM 1515 (Apple Leaves) from the National Institute of Standards and Technologies was used to verify the obtained values. Determination of Zn concentrations in samples were achieved by using the method of calibration curve according to the absorber concentration. Several standard solutions of different known concentrations have been prepared and the elemental concentration in unknown sample was determined by extrapolation from the calibration curve (Table 1).

Table 1. Standard solutions of Zn concentrations to establish the calibration curve.

Standard solution	Concentration [µg/mL]	Mean Absorption
Zn1	0.2	0.0406
Zn2	0.4	0.0915
Zn3	0.6	0,1249
Zn4	0.8	0.1694
Zn5	1.2	0.2452
Zn6	1.5	0.2870
R^2	0.996	

3. RESULTS AND DISCUSSION

The *Zea mays* plants grown on soils contaminated with zinc were well developed, all passing in height over 20 cm in analyzed period. However, the plants were lower in height than the standard sample (sample 0, uncontaminated). In analyzed period was observed that the *Zea mays* plants have a lower height with increasing the zinc concentrations, even the higher amount of zinc stimulated the growth hormone (auxins), which lead at the growth of plants according with [4]. At high zinc concentrations, over normal limit as 12 – 47 mg Zn/kg plant [5] the *Zea mays* is confronted with an excess of zinc ions. Plants contaminated with zinc not been fit into this range (Fig. 1), all having a concentration which exceeded at least 3 times the normal maximum concentration for plants; the sample 0 has a zinc deficiency because the zinc content is below the admitted limit (Table 1).

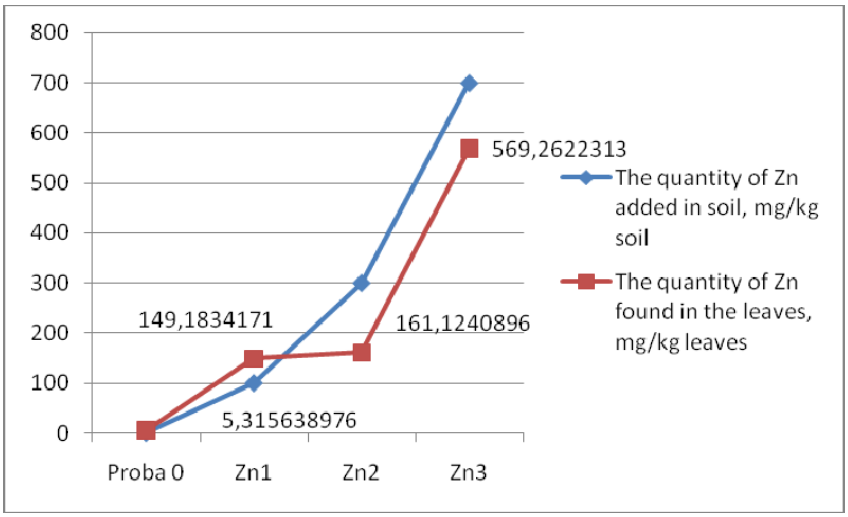


Fig. 1. The quantity of Zn added in soil and found in Zea mays leaves comparative with the sample 0.

Table 1. Concentrations of zinc in *Zea mays* leaves

Samples	**Concentrations of zinc in plant [µg Zn^{2+}/g dried plant]**	**Molar concentrations of zinc from dried plant [mmoles Zn^{2+}]**	**Molar concentrations of zinc from green plant [mmoles Zn^{2+}]**
Normal min.	12.0	0.183486	0.045872
Normal max.	47.0	0.718654	0.179664
Sample 0	5.3156	0.081278	**0.02032**
Zn 1	149.1834	2.281092	0.570273
Zn 2	161.1241	2.463671	0.615918
Zn 3	569.2622	8.704315	**2.176079**

In most of plants the higher concentrations of Zn in soil lead to structural changes and growth problems. According to the changes that occur in plants at different concentrations of zinc, it can emphasize theoretical by three stages during the contamination with zinc.

In the **first stage** zinc is an important microelement for the development of plants. At lower concentrations in cell, this metal catalyzes different enzymatic processes. During the treatment with zinc of *Zea mays* it can observe that, the first interaction phase between Zn^{2+} and cell (Fig. 2) would be characterized by the penetration of sufficient zinc for cell. Therefore the normal reactions to a healthy cell have occurred. This period takes as long as the concentration of zinc in the cell is controlled. Each sample of the contaminated samples pass through this stage, but the duration is higher in sample 0 (remain in this stage until the end of vegetation) and lowest in sample Zn3 (being a high concentration in the medium, ions enter faster in the cell). The concentration of zinc outside the cell is small at sample 0.

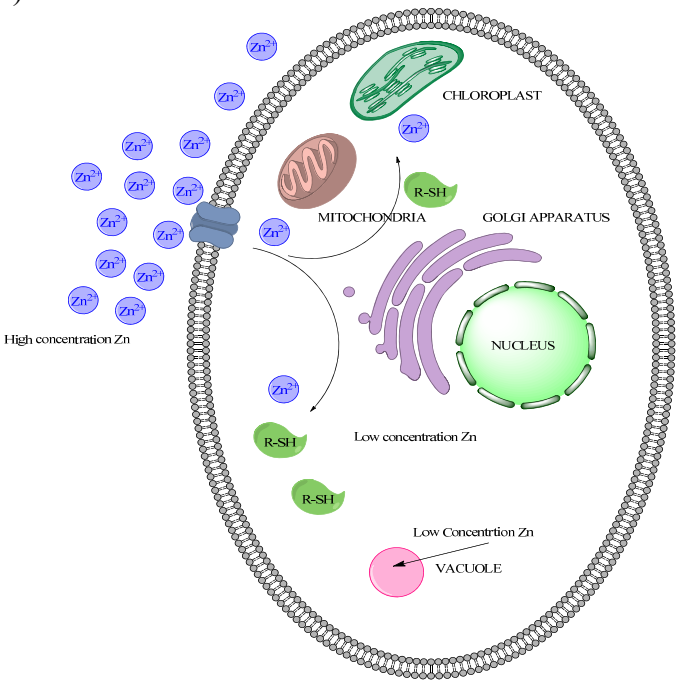
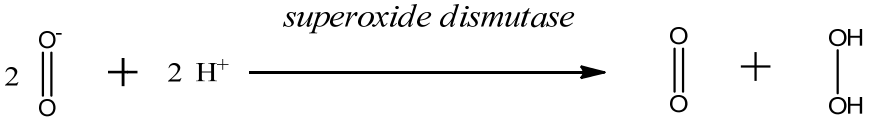


Fig. 2. First interaction phase between Zn2+ and cell.

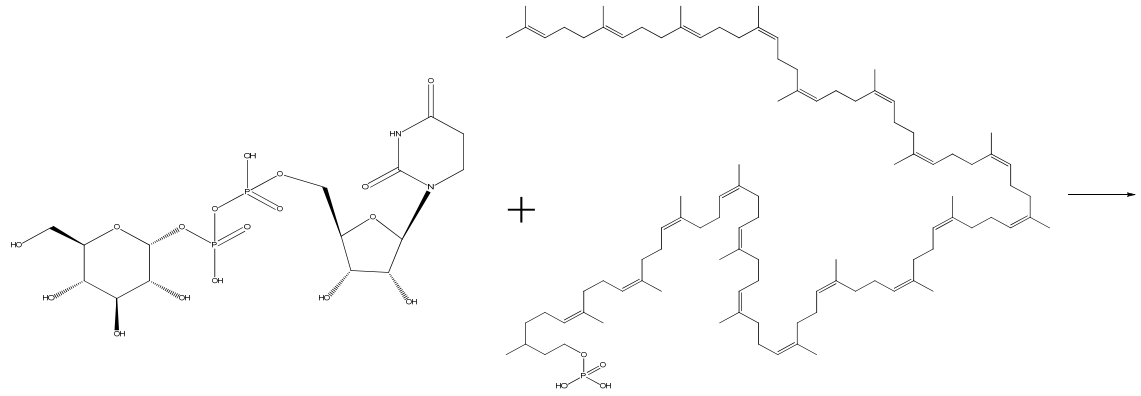
In this stage, the biochemical processes catalyzed by zinc are accelerated. The first enzyme, very important in neutralization of superoxide radicals, *is superoxide dismutase*. It is well known [7, 8] that this enzyme is formed as result of different pollutant factors (UV radiations, heavy metals, pesticide, and other pollutants in air). Superoxide's can destroyed different cellular organs, nucleic acids and proteins. This enzyme is absolutely necessary to *Zea mays* they are an important antioxidant defense in nearly all cells exposed to oxygen. Superoxide dismutase (SOD) catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide according with reaction [9]:



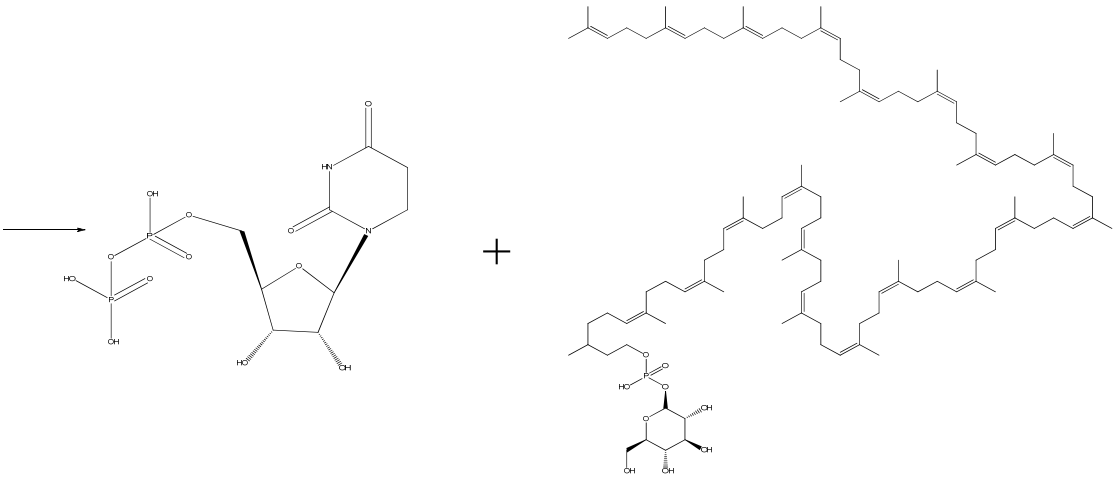
In many studies are presented a lot of superoxide dismutase types at plants, such as superoxide dismutase on Zn-Cu, Mn and Fe [10-12], as well as on Ni, but only at prokaryotes [13]. Zinc deficiency can induce a slowed synthesis of Cu-Zn superoxide dismutase. The absence of this enzyme in the cytosol can cause various unwanted oxidative processes. The sample 0 is possible to have a zinc deficiency, and therefore the plant is more vulnerable to

superoxide radicals. Generally, Cu-Zn superoxide dismutase is founded in cytosol, and the processes from cytosol are affected as well.

Other well known enzyme is *dolichyl-phosphate beta-glucosyl transferase* which participates at dolichyl-diphosphooligosaccharide biosynthesis (important for the synthesis of specific glycoproteins) [14] in according with reactions presented below.



UDP-glucose dolichyl phosphate

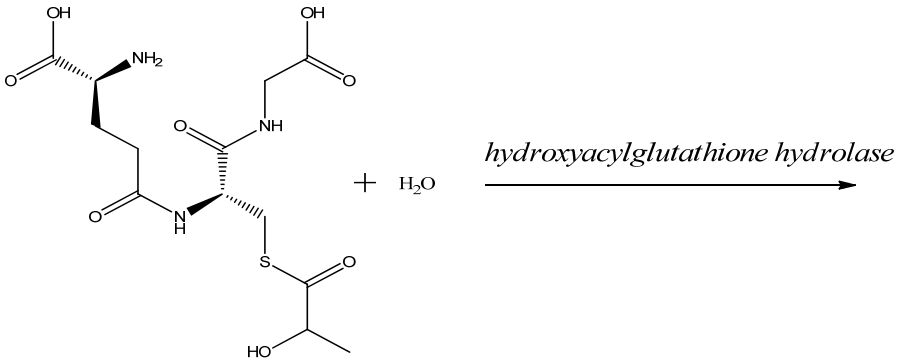


UDP dolichyl beta-D-glucosyl phosphate

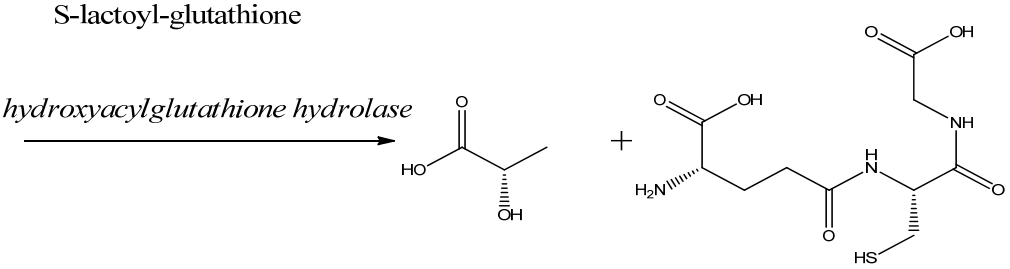
Dolichyl-phosphate beta-glucosyl transferase is activated by a divalent ions, such as Mg^{2+}, Mn^{2+}, Ca^{2+}, Co^{2+}, Zn^{2+} (in descending order of activation) [15]. Even if the plant has a zinc deficiency, the reaction can not be affected by the presence of other ions.

Hydroxyacylglutathione hydrolase [16-19] is an enzyme which participates in detoxification of plant by methylglyoxal and catalyzes the second stage of transformation of S-lactoyl-glutathione in (R)-lactate according with reactions presented below:

This enzyme is dependent of zinc. In absence of zinc the S-lactoyl-glutathione is transformed in methylglyoxal [20]. In analyzed samples it is possible as sample 0 to have a higher content of toxic methylglyoxal, due that the sample 0 has a lower concentration of zinc, below the normal admitted limit.

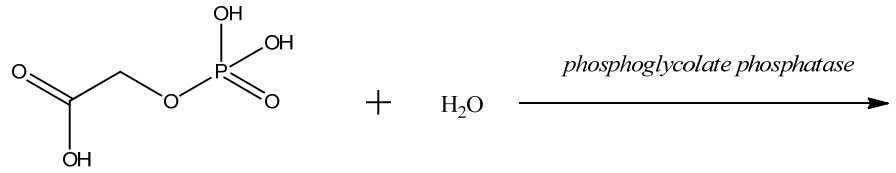


S-lactoyl-glutathione

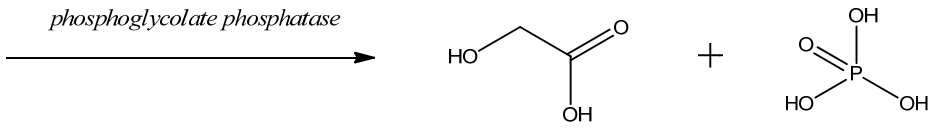


(*R*)-lactate glutathione

Other important enzyme with is activated by the Zn^{2+} is *phosphoglycolate phosphatase*. This enzyme participates at dephosphorylation reaction of 2-phosphoglycolate at glycolate, an important phase by photorespiration (cycle Calvin-Benson-Bassham) [21-23]. It is well known that zinc is an activator of this enzyme, but lower than Mg^{2+} [24]. If the amount of zinc is lower then is hard to suppose that the enzyme is inactivated.

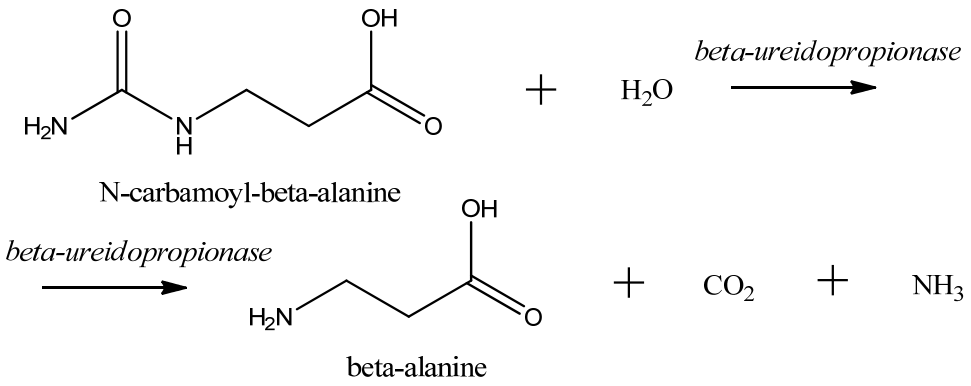


2-Phosphoglycolate



glycolate phosphate

Beta-ureidopropionase is an enzyme existing in *Zea Mays* which catalyzed the hydrolysis reaction of linear amides. In *Zea mays*, such reaction occurs in uracil metabolism. The zinc activated the enzyme; in absence of zinc, hydrolysis reaction has a lower yield [25, 26]. At sample 0, it is possible as β-alanine synthesis to be affected, so the proteins may have a lower content of β-alanine.



The **second stage** is characterized by excessive increase of zinc concentration in the cytoplasm. This phenomenon occurs due to increased mobility of Zn^{2+}, which passes easily through by cell membranes. Zinc passes through by cell membrane, because is fixed very little by the polygalacturonic acid which is present in membrane. Polygalacturonic acid fixes the heavy metals in the following order: Pb> Cr> Cu> As> Zn. The mobility of zinc is higher than calcium, so it is expected that at an excess of zinc in the cell, to be held a calcium deficiency [6]. Many biological processes are affected due to total or partial inhibition of several enzymes as is shown in Fig. 3.

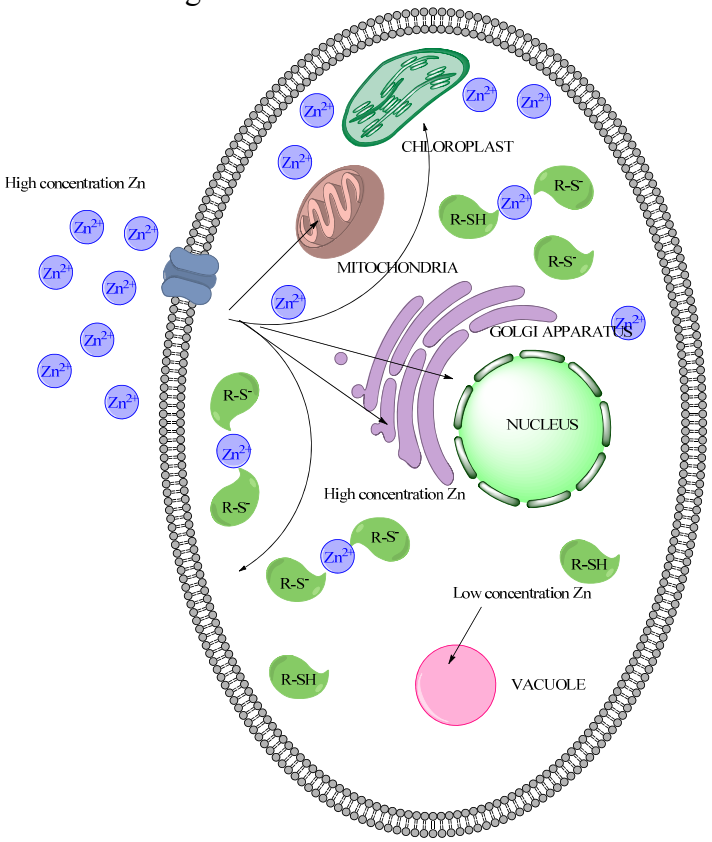
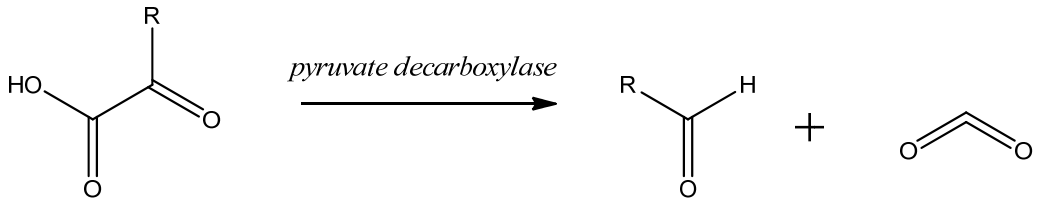


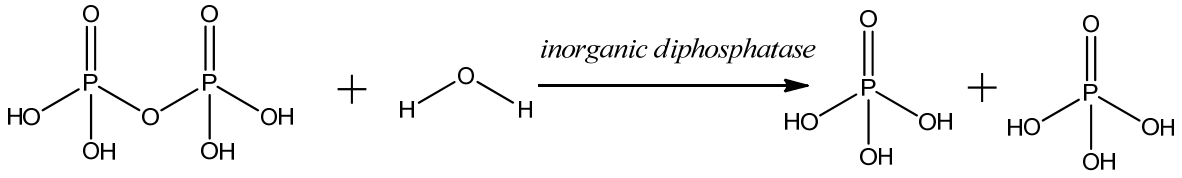
Fig. 3. Second stage interaction between Zn and plant cell.

In this stage, the first enzyme, which can be inhibited by the amount of zinc, is pyruvate decarboxylase. This enzyme participates in the first decomposition phase of pyruvate to acetaldehyde [27]. Zinc is a weak inhibitor of this reaction, much weaker than Hg^{2+} (which completely inactivates) [28]. Partial inhibition of the reaction is possible in all samples contaminated with zinc. Mostly is affected the sample Zn3. The enzyme catalyzes the degradation reaction of leucine and valine, as well, according to reaction as folows:



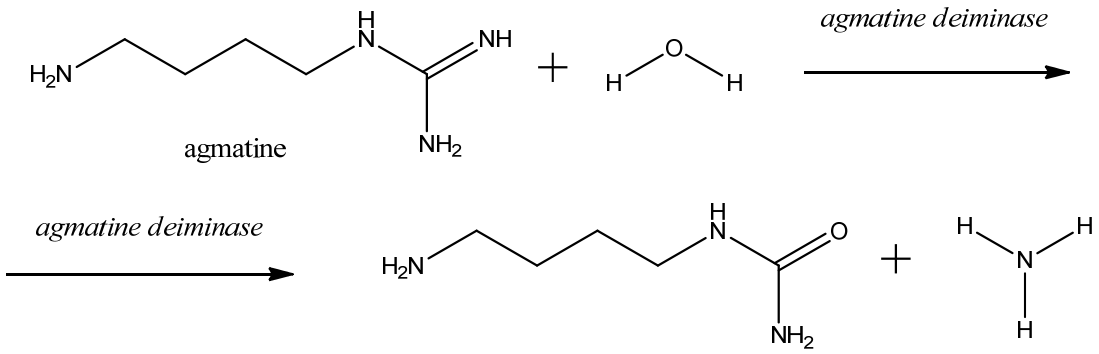
2-oxo acid aldehyde

Inorganic diphosphatase is an enzyme which participates in energetic metabolism according with reaction presented below:



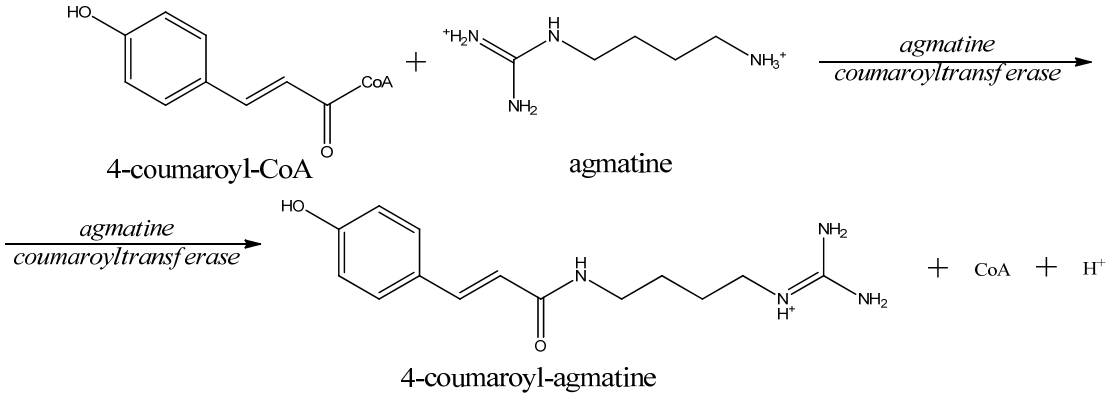
It is well known [29] that zinc has a weaker influence on the enzyme. If the enzyme would still be inactivated, then the reaction can be catalyzed by other enzymes, such as: alkaline phosphatase, acid phosphatase or glucose-6-phosphatase.

Agmatine deiminase participates at transformation of L-arginine in putrescine, by catalyzing the intermediary reaction of N-carbamylputrescine formation from agmatine [30].

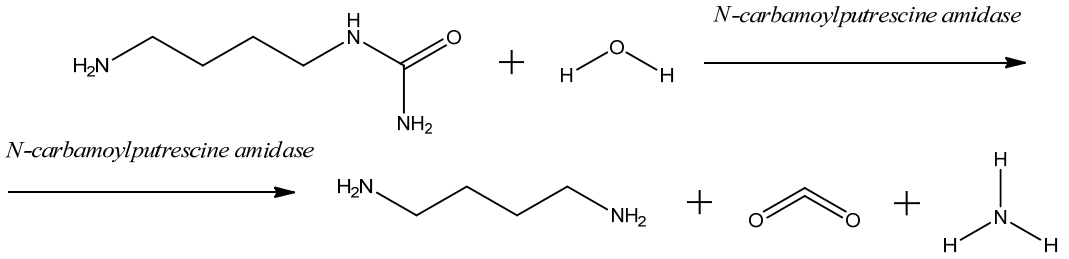


N-carbamoylputrescine

Putresceine is a polyamide which participates at a lot of biological processes. This polyamide forms some compounds with nucleic acids, establishing cell membranes and has a stimulating role for different enzymes. At higher zinc concentrations can appear the risk as reaction to be inhibited (at 1 mM Zn^{2+} the inhibition is until 85%) [31]. According with these data in analyzed samples, Zn1 and Zn2, were determined concentrations under 1 mM Zn^{2+}, so the agmatine deiminase is not inhibited until 85%, but in sample Zn3, with 2 mM Zn^{2+} is possible as reaction to be total inhibited. Some compounds, such as 4-coumaroyl-agmatine and ferulaylagmatine have an antifungal effect. These compounds compensate the fact that cell membranes are weaker due to inhibition of the formation reaction of putrescine [32-34]. Thus is formed other compounds, such as 4-coumaroyl-agmatine (and ferulaylagmatine) as in reaction:

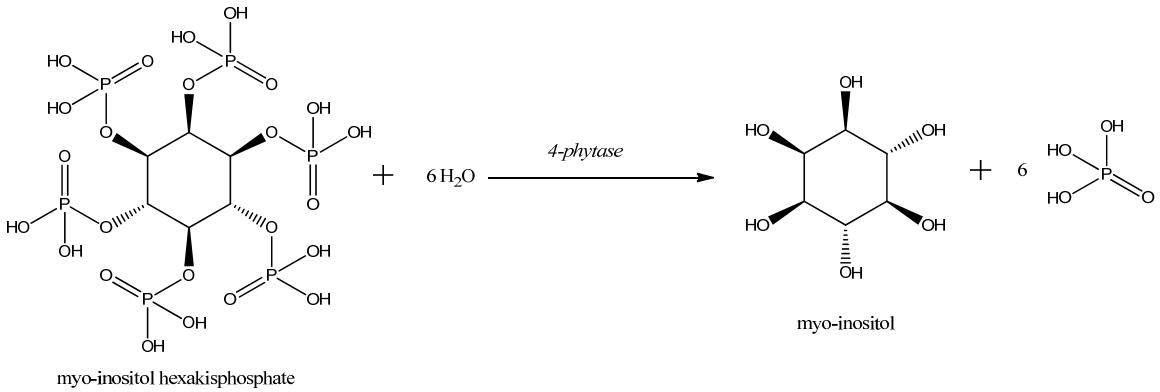


N-carbamoylputrescine amidase is an enzyme which catalyzed the final phase of chain transformation reactions of arginine in putresceine (in fact that the N-carbamoylputrescine passes in putrescine) according with the following reaction:



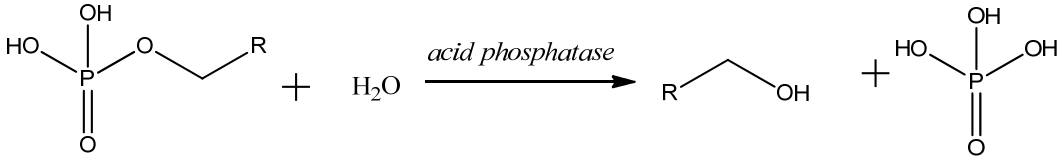
Zinc has a strong negative action on the reaction [35], which theoretically would lead to an accumulation of N-carbamoylputrescine, but this does not happen because the previous reaction is also inhibited. Therefore decreases not only the probability of putrescine formation by this reaction, but the ability to accumulate N-carbamoylputrescine as well.

4-Phytase is an enzyme which can catalyze some fundamental processes as ATP dephosphorylation, an important process which is initiated by many enzymes. This enzyme is important as well, in chain decomposition reactions of myo-inositol hexakisphosphate in seeds [36]. Myo-inosol hexakisphosphate, named phytic acid, concentrated almost all the phosphor from seeds (80% of phosphor is in phytic acid [37]). This acid can added different mineral [38, 39] which are important of plants, because during of the germination, the embryo takes up almost all minerals and phosphorus from this compound.



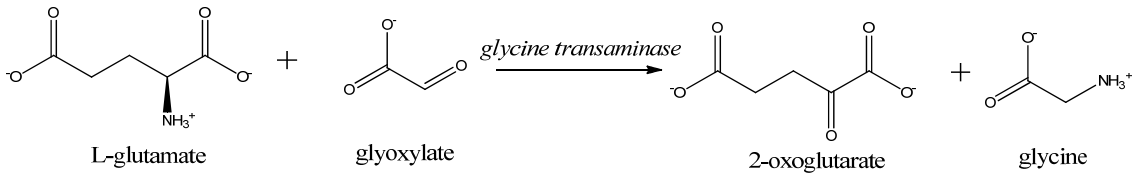
Zinc has a strong inhibitor effect on the dephosphorylation reaction of myo-inosol hexakisphosphate and thus decreases the power of seed germination [40, 36]. The seeds obtained on strong contaminated soils would greatly decrease the power of seed ingemination due to the blockade of this reaction. During of seeds germination is very important the germination medium. The higher amount of zinc can lead to the inhibition of reaction as well. The young plant records an important deficit of phosphor and thus the growth rate of plant decrease. Between all analyzed samples, the sample Zn3 was germinated at the latest. Zinc has a strong influence on 4-phytase enzyme and from this point of view, in the sample Zn3 can suppose that the phosphor from phytic acid could not be used for biochemical processes.

Acid phosphatase is an enzyme with the same function as 4-phytase, participates at dephosphorylation reactions of organic esters of phosphoric acid [41].

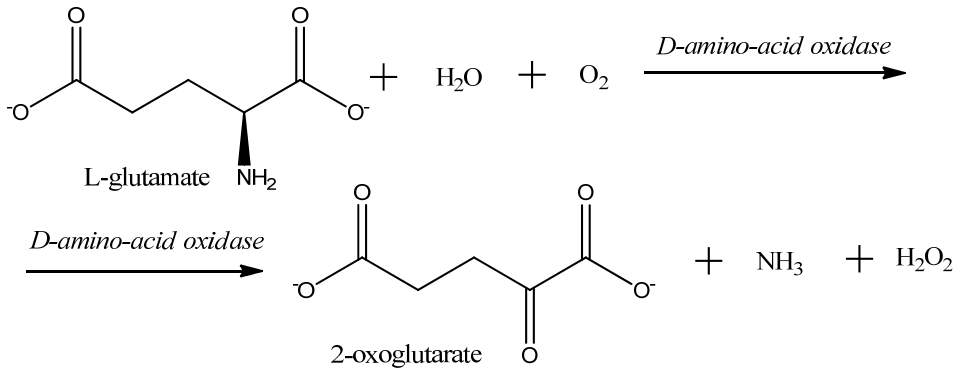


This enzyme participates at different processes such as: dephosphorylation reaction of NADPH at NADH, recycling of phosphor in plant, regeneration of cell walls, riboflavin metabolism. Zinc affects these processes, producing an inhibition of the enzyme at a rate of 54.8% at concentration of 1 mM [42]. The contaminated plants with zinc have the cell walls less developed due to damage process of synthesis of β-glucans [43].

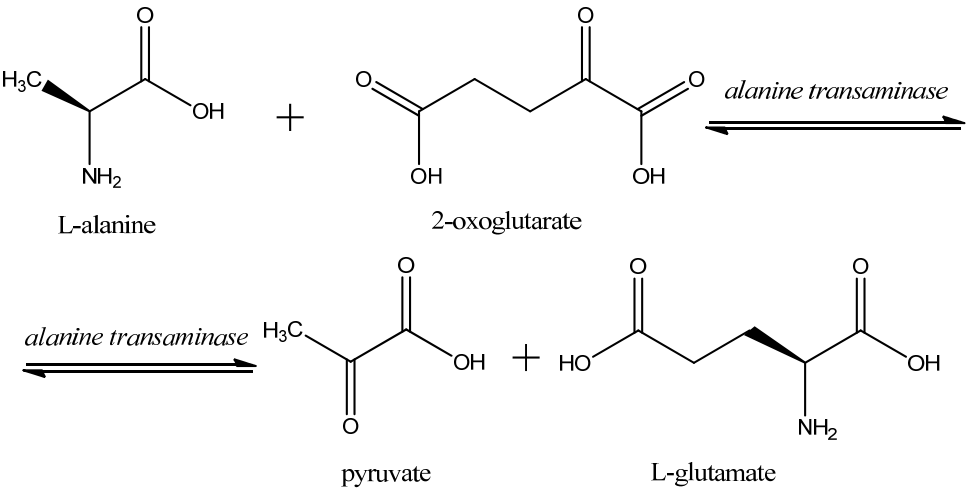
Glycine transaminase is an enzyme which catalyzes reaction between glycine and 2-oxoglutarate, when resulted glyoxoglate and L-glutamate according with reaction:



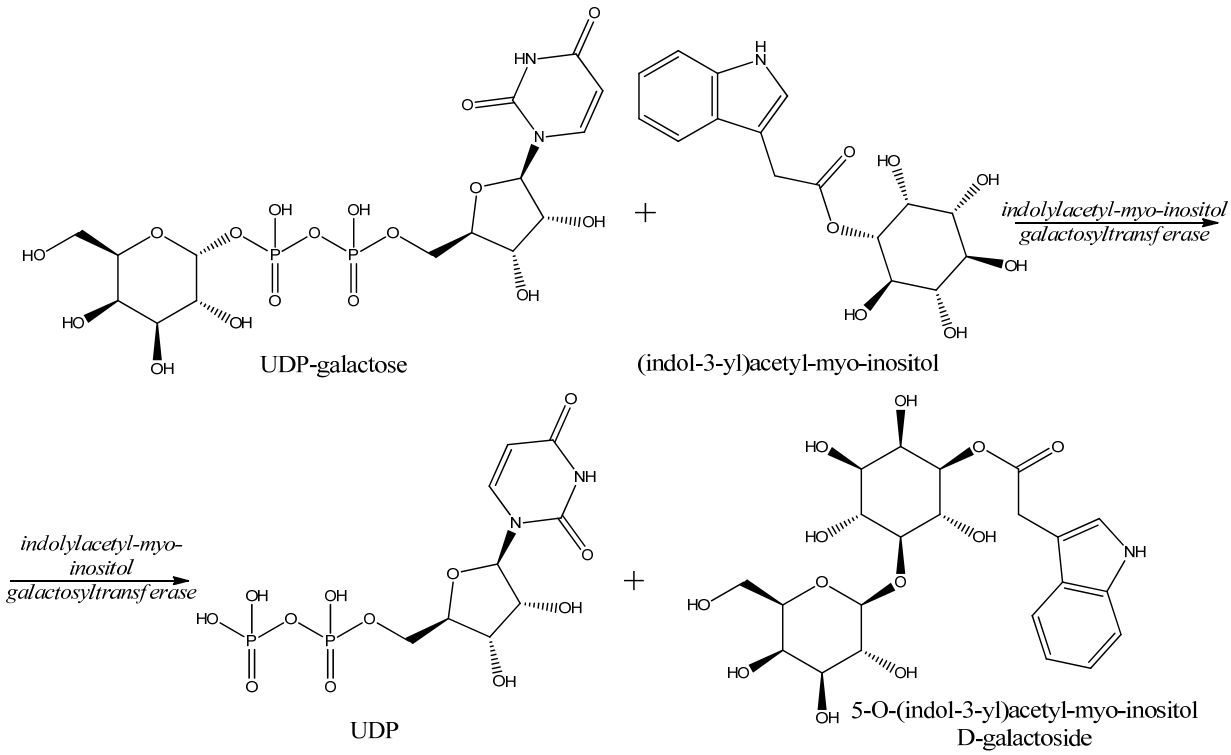
This reaction is a transfer reaction of amino group, from glycine, serine and tronine metabolism, and the reversed reaction is part of the photorespiration process. It is well known that [44] zinc inhibit reaction with 25% at 1 mM Zn^{2+} concentration. In this respect the transformation of glycine in glyoxylate can take other way. D-amino-acid oxydase enzyme can lead to glyoxylate, with formation of H_2O_2 and NH_3, which can operate negative on plant [45]. Thus the sample Zn3 must have a higher content H_2O_2 și NH_3 or other products resulted from reactions between these and other compounds.



Alanine transaminase is an enzyme which catalyzes the synthesis reaction of L-alanine from pyruvate. This reaction can realize in reverse being catalyzed by the same enzyme. Thus, it occur the equilibrium adjustment between pyruvate and L-alanine, according to plant needs [44]. The zinc ions inhibit the enzyme with 75% at 1 mM Zn^{2+}, Thus at samples Zn1 and Zn2 the reaction catalyzed by alanine transmitase must be partially inhibit, and at Zn3 sample reaction is even total inhibited.

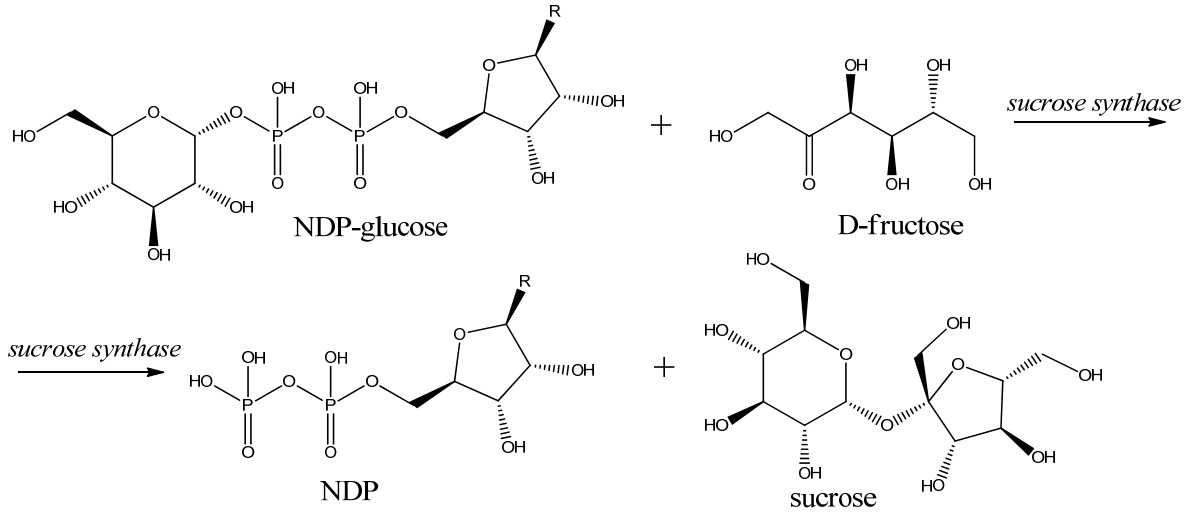


Indolylacetyl-myo-inositol galactosyltransferase is an enzyme which participates in indol-3-yl-acetic acid (auxin) metabolism, which is part in growth hormones of plant. Indol-3-yl-acetic acid is absolutely necessary in the division and growth process of cell in seeds.

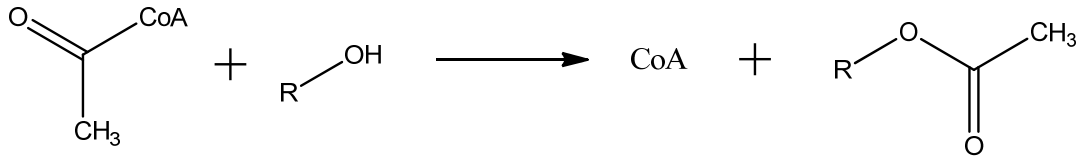


It was observed that on enzyme some ions such as Ca^{2+}, Mn^{2+}, and Mg^{2+} can act positive. The most powerful effect it has Ca^{2+}. The Zn^{2+} produces a yield so small (five times lower than Ca^{2+}) that can be considered to have an inhibitory effect [46]. However, appear the possibility to form the 3-yl-acetyl-myo-inositol L-arabinoside due to indolylacetyl-myo-inositol galactosyltransferase enzyme.

Sucrose synthase catalyze the synthesis reaction of sucrose from NDP-glucose and D-fructose [47]. The research [48] shown that ions of zinc have a strong inhibitory effect on this reaction [48]. In all analyzed samples, which are treated with zinc ions, is possible that synthesis reaction of sucrose to be impaired. The Zn3 sample has the lower content of this disaccharide.

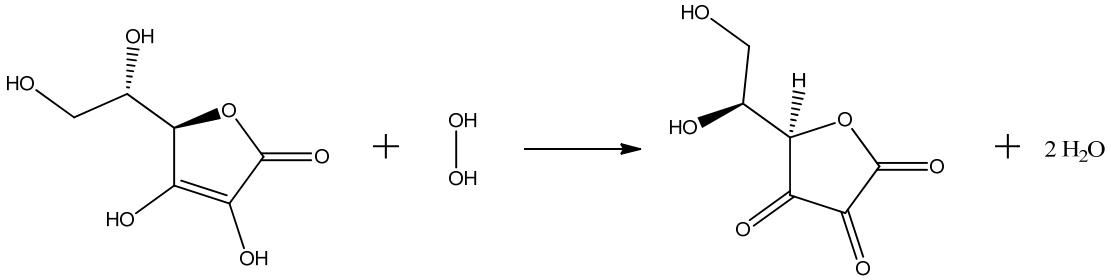


Histone acetyltransferase is an enzyme which catalyzes the obtained reaction of CoA from acetyl-CoA [49].



The inhibition of reaction occurs at 5 mM Zn^{2+} [50]. None of the analyzed samples does not have higher zinc content; thus that it can suppose that the reaction is inhibited.

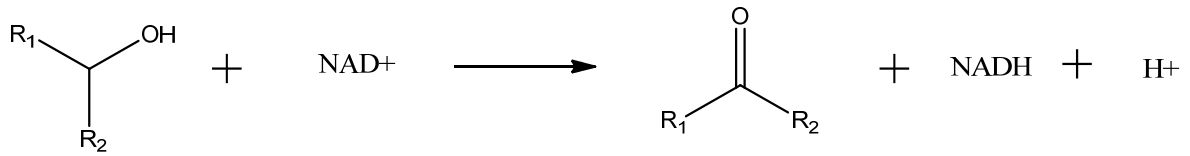
L-ascorbate peroxidase is an important enzyme in ascorbate metabolism and for neutralization of hydrogen peroxide, according with reaction below:



L-ascorbate dehydroascorbate

The higher concentrations of zinc decrease the activity of this enzyme, resulting lipid peroxides ascorbate and dehydroascorbate [51].

Alcohol dehydrogenase is an enzyme as well, that catalyze dehydrogenation reaction of alcohols at aldehydes and ketones. This enzyme is dependent by zinc and at higher concentration of zinc can be inhibited [52].



In the **third stage** (Fig. 4), to achieve high levels of zinc in cytoplasm, are initiated various detoxification processes of the cell. In the cell starts the synthesis of phytochelates and metalotioneines. It blocks the free metal ions or transporting them to the vacuole (phytochelates) [53]. This stage is characterized by mechanisms which have the principal scope the cell remediation. Other remediation mechanism of cell is the using of zinc in different biochemical processes as substituted of different ions.

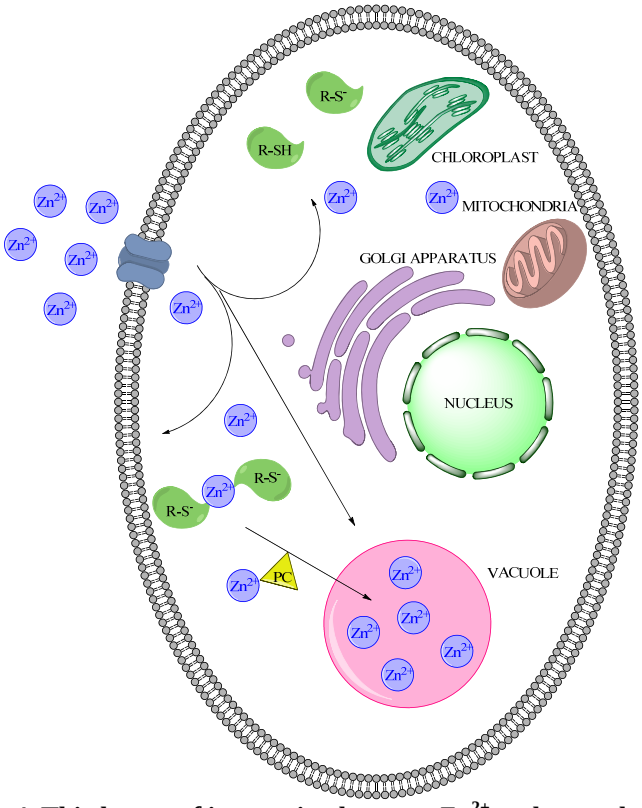


Fig. 4. Third stage of interaction between Zn^{2+} and vegetal cell.

4. CONCLUSIONS

How simple it seems a small leaf from a plant which occupies an enormous area. Sometimes we remain so enthusiastic about what we do that we begin to think that we have control over everything. And we do not have ... That little leaf has the control over almost everything that is alive, thanks to the phenomenon of photosynthesis. Thousands of biochemical processes are struggling to catch CO_2 and chemicals to release O_2. It's hard to imagine that they can have the control of all these processes, especially when it comes to cell. So ordered is this biological system that even disturbed by certain factors (e.g. pollution), it returns to normal within a period of time. Zinc, an essential trace mineral, can become a powerful toxin for plant and all acting the concentration. Sample Zn3, which has accumulated the largest amount of metal, is in critical condition. Metal ions have so much disturbed the biochemical processes in the plant that most of the energy required to the plant passes to detoxify it leaves. Obviously, it will record a lower growth of *Zea mays* plant in all respects. Samples Zn1 and Zn2 are the samples with intermediary concentrations of zinc, the administration effects being observed less, especially in Zn1.

The importance of maintaining the zinc concentrations optimal to development of plant in environment is very important. This is the only way to ensure the equilibrated development in all cultures (including *Zea mays*). The problem is not only that production efficiency may decrease, but the quality of this production is very low.

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