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INFLUENCE OF THE PHOTODYNAMIC EFFECT ON THE TISSUE WATER EXCHANGE IN SOME MONOCOTYLEDONOUS AND DICOTYLEDONOUS PLANTS

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Summary. The influence of some early precursors of chlorophyll biosynthesis such as δ-aminolevulinic acid (ALA) and glutamic acid (GA), together with the modulator of chlorophyll synthesis – 2,2´-dipyridyl (DP) on dynamic of the tissue water exchange was studied as a consequence of photodynamic action. The accumulation of porphyrines, caused by the combination of chlorophyll precursor and DP, has a photodestructive effect on dicotylednous plants. One of the most probable mechanism of this action is the destruction of the cell membranes, which causes increased water transfer trough the membrane and this might be the reason of a total change in water exchange in the tissues in some green dicotyledonous plants. The plants respond to the water deficit, caused by the destruction of the cell membranes, by lowering their osmotic potential and an increase of the amount of the inflow water from the hypotonic solutions, and the decrease of the amount of the outflow water in hypertonic concentrations. The results from water tissue exchange show that this effect is very little in monocotyledonous plants. The dicotyledonous plants are more sensitive to such treatment showing mostly an increased water loss from the treated leaves.

Keywords: δ-aminolevulinic acid, 2,2´-dipyridyl, glutamic acid, photodynamic processes, water exchange.

Abbreviations: ALA – δ-aminolevulinic acid, DP – 2,2´-dipyridyl, GA – glutamic acid, Pchlide – protochlorophyllide, Chlide – chlorophyllide, Chl – chlorophyll, DV – divinyl, MV – monovinyl.

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Introduction

The synthesis of chlorophyll (Chl) in the plants follows a long pathway of metabolic reactions, which includes a number of precursors and intermediates of the glutamic acid (GA) serving as a 5-carbone backbone for the synthesis of δ-aminolevulinic acid (ALA). It is well known that the exogenous supply of ALA causes a strong increase in protochlorophyllide (Pchlide) amount as well as in other chlorophyll precursors (Rebeiz et al., 1984). The Pchlide accumulated in such conditions is in a photo inactive form ($Pchlide_{653}$) and its conversion to chlorophyllide (Chlide) is very slow and by an intermediate changes to its photoactive form of $Pchlide_{657}$ (Granick, 1961; Beale and Weinstein, 1991). The irradiation of such plants with accumulated $Pchlide_{653}$ causes photooxidative stress, which is manifested as leaves damage, decrease of the leaf turgor and pigment chloroses (Rebeiz et al., 1984). The accumulated porphyrines act as photosensitizers, which eventually lead to plant cell destruction (Chakraborty and Tripathy, 1992). Such compounds are mentioned as photodynamic herbicides, due to their possibilities to be used in the agriculture practice as specific herbicides (Rebeiz et al., 1984). In the practice of using such combination, it was found that there are substances, which can increase the accumulation of photooxidating tetrapyroles and they are called modulators of chlorophyll synthesis. Such is the action of 2,2´-dipyridyl (DP), which induces porphyrines synthesis in green plants (Toneva et al., 1997).

A similar way to achieve the similar photodynamic effect is to use the immediate ALA precursor, that is the GA. There are some investigations of the effect of such replacement of ALA, showing that it can also cause a considerable photodynamic action through accumulation of porphyrines as chlorophyll precursors and especially the photoinactive Pchlide (Koleva et al., 1995; Toneva et al., 1997). There are some brief indications for the visual dehydration of the plants, sensitive to such treatment (Rebeiz et al., 1984, 1988). It is believed that in the light the accumulated porphyrins photosensitize the formation of singlet oxygen, which is very strong oxidant (Becerril et al., 1992). The active singlet oxygen forms accumulated in the plant tissues, show a complex influence on the total metabolism by inhibition of photosynthesis, inactivation of key enzymes of oxidation path of amino acids, damage of the cell membranes due to the oxidation of the membrane proteins and nonsaturated fatty acids, and finally all reactions lead to the disintegration of the plant tissues (Anderson et al., 1990; Becana et al., 1998; Knox and Dodge, 1985; Mayfield et al., 1986; Navari-Izzo et al., 1994).

In normal conditions, the water inside the cell is defined in terms of its free energy content or the water potential (Ψ_w). The water potential in the cell is dependent on the osmotic potential (Ψ_s) and the turgor pressure (Ψ_t). Some factors in the cell as the content of solutes (Ψ_w) and the pressure of the cell content on the wall (Ψ_w) decreased the water potential. It is also dependent on the matric potential, or the binding water to the cell surfaces (Ψ_m). The membrane permeability is also involved in the control of the cellular water potential and turgor (Ann Brey, E., 2001). Since the permeability of the cell membranes is in a close relation with their physiological state,

our interests was directed to follow the changes of water exchange as a direct consequence of the cell membranes permeability caused by the oxidative stress from tetrapyroles accumulated in treated plants.

Materials and Methods

The experiment was conducted with oat (*Avena sativa* L.) (monocotyledonous), and vegetable marrow (*Cucurbita moschata* Duch.) and cucumber (*Cucumis sativus* L.) (dicotyledonous).

Plants were grown in a growth chamber with 12 h light/12 h dark cycles at a light intensity of 48 $W.m^{-2}$, at 22–25 ºC, and 60–70% relative humidity. The treatment was done by sprinkling the leaves with solutions of 5 mM ALA, 15 mM DP, 20 mM GA, on different stages of their development as follows: oat – on the phase of third leaf; cucumber and vegetable marrow – on the phase of fourth-fifth real leaf. After the treatment the leaves were incubated in darkness for 17 hours. The dynamic characteristic of the tissue water exchange and the water content were measured after the dark period and after 24 hours of irradiation, followed by dark incubation. The intensity of irradiation of the plants after different treatments was 48 $W.m^{-2}$.

The dynamic characteristic of the tissue water exchange was performed by Gusev (1960). The method is based on the use of sucrose solution with different molarities (0.1 M = 2.675 bar; 0.2 M = 5.360 bar; 0.4 M = 11.257 bar; 0.6 M = 18.005 bar; 0.8 M = 25.878 bar and 1.0 M = 35.058 bar) and the concentration is measured refractometrically. The leaves segments were immersed in the sucrose solutions for 3 hours to reach the equilibrium of water concentration between solutions and the tissue. The amount of the water, which was detracted from the leaves to the sucrose solutions, was referred as to "free" water and it was calculated as follows $X = (A – B).T/B$, where A is the primary concentration of sucrose solution (%); B is the end concentration of sucrose solution (%); T is the primary weight of 2 ml of the sucrose solution.

The total water content in the plant tissue was measured after a total drying of the leaves at 105 ºC for 6 h. The difference between the total water content and the "free" water was referred as to "bound" water (Y). The amounts of the "free" water (B_x) and the "bound" water (B_y) were calculated as a percent from the total water content in the leaves. The amount of "free"water, calculated in the different sucrose solutions, plotted against the total water amount in the leaves was referred to dynamic characteristic of the water exchange.

Results

Immediately after the dark incubation there is no difference in the water content of the leaves, treated with ALA, GA and DP). Irradiation of the oat leaves also do not change the total water content, but the dicotyledonous plants show a substantial dec-

rease (25–60%) of the water which was observed in all treatments. The results of these investigations are presented elsewhere (Toneva, Minkov 2001).

The changes in the dynamic characteristics of the water exchange differed from the changes of the total amounts and they were specific for both plant types used for experiments. Figure 1 shows the dynamics of water exchange in oat leaves, treated with ALA, GA and DP and their combinations. After 17 hours of dark incubation there was not a considerable difference between treated and control plants (Fig. 1A). In all the experiments, because the water potential of the hypotonic sucrose solutions was greater than the water potential of the cells, the plant cells took up water. The increased sucrose concentration lowers the solution water potential, draws water out from the cell, and thereby reduces the cell's turgor pressure. The amount of detracted water from the highest concentration (1.0 M) in all cases of different experiments and the control plants was in the range of 60.5–63.5%.

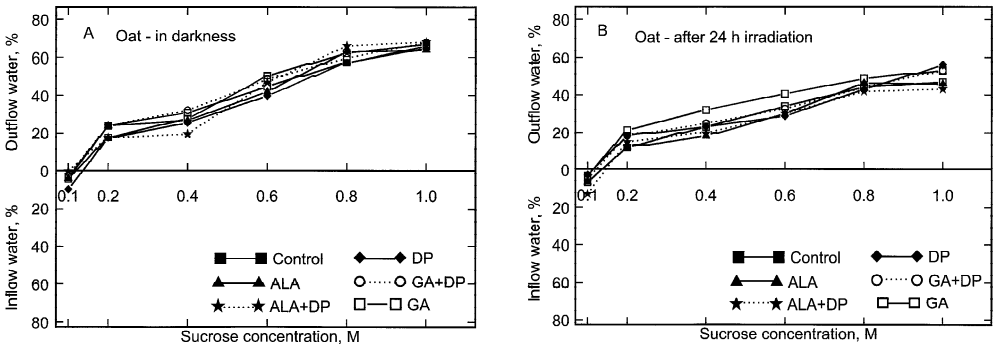

Fig. 1. Dynamic of the tissue water exchange in oat leaves, treated with δ-aminolevulinic acid (ALA), glutamic acid (GA) and 2,2´-dipyridyl (DP), after 17 h of dark incubation (A) and 24 h of irradiation (B).

After 24 hours of irradiation (Fig.1B) there was still no difference between the treated and the control plants, taken immediately after the dark incubation. There was a similar amount of water detracted from the leaves as in highest sucrose concentration.

The results from treated of vegetable marrow plants are presented in Fig. 2. After 17 hours of dark incubation with ALA, GA and DP the water exchange in different sucrose concentrations did not change substantially (Fig. 2A). The water uptake from the hypotonic concentration was about 9,8% in the leaves treated with GA (5% in control plants), and the water detracted from the treated with GA+DP leaves was about 60% (65% for the control plants and the rest of the treated plants).

After 24 hours of irradiation (Fig. 2B) difference between the treated and control plants was observed. The plants treated with the photodynamic combination of ALA+DP showed the highest amount of water taken from the lowest sucrose concentration – 33.2%. The treatment of the plants with DP only decreased this amount with 5.5% compared with control plants. In concentration of 0,4 M of sucrose the water outflow in the plants with the combination of ALA+DP was 15,3% and under the

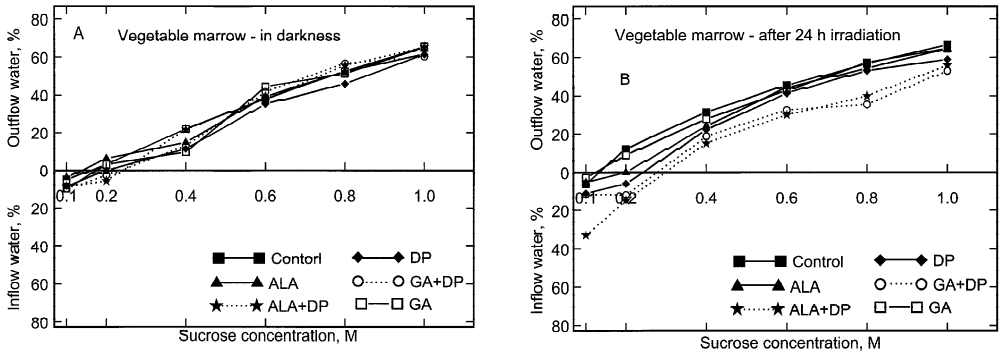

Fig. 2. Dynamic of the tissue water exchange in vegetable marrow leaves, treated with δ-aminolevulinic acid (ALA), glutamic acid (GA) and 2,2´-dipyridyl (DP), after 17 h of dark incubation (A) and 24 h of irradiation (B).

treatment with GA+DP was 18,8%. At the same time the water outflow in the control plants was 31,6%. This tendency is kept also in other concentrations of sucrose solutions, shown in 0,8 M sucrose where the difference between the treated and control plants was about 16%.

The effect of treatments on the tissue water exchange is shown in Fig. 3. After the dark period of incubation (Fig. 3A) the exchange of water amounts were close to all used combinations and in all concentrations of sucrose. The tendency was similar to this, seen in the experiments with vegetable marrow. The irradiation of the treated plants (Fig. 3B) caused bigger differences between treated and control plants. In the hypotonic sucrose concentration the treated leaves took up the highest amounts of water. In the variant of treatment with ALA + DP this amount was the highest – 68,3% which is almost 20 times higher than the control plants. Similar, but nevertheless lower effect had the DP alone and the GA+DP – 19,5%. Close to the results obtained with vegetable marrow, the cucumber leaves treated with ALA+DP and GA+DP showed

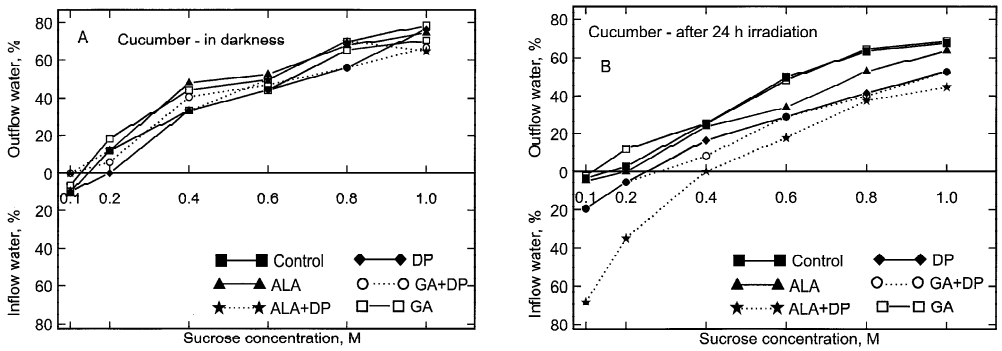

Fig. 3. Dynamic of the tissue water exchange in cucumber leaves, treated with δ-aminolevulinic acid (ALA), glutamic acid (GA) and 2,2´-dipyridyl (DP), after 17 h of dark incubation (A) and 24 h of irradiation (B).

the lowest amounts of detracted water in higher sucrose concentrations. The plants, treated with GA alone did not differ from the control plants.

Discussion

The chlorophyll synthesis is a long and sophisticated chain of biochemical reactions with a complicated enzyme system. In the green plants no precursors are accumulated since the equilibrium is strongly shifted to the chlorophyll formation. High amounts of porphyrines (Proto, Mg-ProtoME and Pchlide) were accumulated in darkness after treatment with ALA, GA and DP which strongly influences the water amount in the irradiated leaves, especially the water considered as "free" water. This water is less bounded to hydrophilic protein molecules and is easily detracted from the cells, by using solutions with higher osmotic concentrations (Fig. 1–3). This is most probably caused by the photooxidative processes after porphyrines accumulation (Averina et al., 1989; Chakraborty and Tripathy 1992; Koleva et al., 1995; Koleva and Toneva, 1998; Rebeiz et al., 1984, 1988; Rebeiz et al., 1987; Toneva et al., 1997). There are some indications, showing that the accumulation of monovinyl (MV)- and divinyl (DV) -protochlorophyllides are among the most important cause for cell destruction in the treated leaves (Chakraborty and Tripathy 1992; Rebeiz et al., 1984, 1988). The oat belongs to greening group Dark MV/Light DV (Rebeiz, 1988). During the night, they accumulate mainly MV–protochlorophyllide. Under daylight, the plants shift back to a DV–protochlorophyllide. The treament of oat plants with ALA, GA, and DP probably does not change the type of the chlorophyll precursors.

The studied dicotyledonous plants belong to the greening group described as Dark DV/Light DV. Normally in the dark phase of the photoperiod these plants synthesize DV-forms of precursors (Pchlide). Later in the daylight the chlorophyll synthesis goes through a pool, enriched in DV-Pchlide (Rebeiz, 1988). When treated with ALA, GA and DP, the plants from this greening group are forced to synthesise the "wrong" type of tetrapyroles (MVl) which makes them sensitive to the accumulated sensitizes which causes the lethal effect, shown in our studies.

The treatment with ALA, GA and DP showed high resistance of the tissue water exchange of monocotyledonous plants. Some suggestions about this had been discussed earlier (Rebeiz et al. 1987, Averina et al. 1989).

The treatment of the monocotyledonous plants did not change the total water amount in their leaves nor immediately after the dark incubation, neither after 24 hours of irradiation (Toneva, Minkov 2001). Almost the same pattern of the water exchange parameters was seen, showing that there had been not much changes of all tissue water turnaround in the conditions of higher precursor content. The irradiation of the treated dicotyledonous plants changed both the total water content and different water fractions, showing both a severe water deficit and substantial change in the water exchange, expressed as a changed ratio between the "free" and "bound" water. The

total water quantity was low which can explain the higher amount of water taken from the sucrose solutions with lower molarities (Fig. 2 and 3).

The plants respond to the water deficit by lowering their osmotic potential through the accumulation of osmolytes as sucrose (Smirnoff, 1993; Pelah et al., 1997a,b) and proline (Meier et al., 1992). The production of osmolytes may have multiple function and also inhibit oxidative stress (Bohnert and Jensen, 1996). Such a behavior can explain why the cross point of the curve of water exchange with the abscissa, showing an isotonic concentration equal to the osmotic potential of the cells, is shifting to a higher concentration in damaged monocotyledonous plants (Fig. 1a,b) and dicotyledonous plants (Fig. 2b, 3b), compared with the nontreated plants. This shift is most probably due to the elevated concentration of osmotic substances, caused by the oxidative stress. This, together with the highly increased water deficit in cucumber and vegetable marrow, leads to a decrease of the water potential causing an increase of the amount of the water taken from hypotonic sucrose solutions, and the decrease of it in hypertonic concentrations. The lower water amount taken from the damaged leaves in hypotonic solutions are due to the lower total water content and not to the higher water keeping ability of these plants (Toneva and Minkov, 2001). This tendency was relatively better expressed in cucumber plants, pointing to existence of species specificity among the dicotyledonous plants (Fig. 3). The lower amounts of “free” water means that the total water loss in photodynamic conditions concerns mostly the less bound water fraction in the plants tissues (Fig. 2), but the more severe destruction of the plants membrane in cucumber plants (Fig. 3) influences also the “bound” water fraction. It seems that the effect of the photodynamic destruction of the cells depends on the rate with which the tetrapyroles are synthesized under the influence of ALA, GA and DP and the pace of their destruction in light. It is well known (Becerril and Duke 1989; Becerril et al., 1992) that those features are specific for different species, which synthesize different amounts of MV and DV chlorophyll precursors (mostly Pchlide). On the other hand the found specificity in water changes can be due to the anatomic features of their leaves, which can have big differences between monocotyledonous and dicotyledonous plants. It was established that the water amount and turnover can be the first indications of the photodynamic damage of plants under the influence of elevated amounts of tetrapyrols accumulated in the cell. The rapid change in water deficit shows that the plant membranes is the first to be damaged in the plants cell, resulting in higher ion exchange (Toneva, Minkov, 2001) and water amount changes in treated leaves. This can have a practical implementation in rapid testing the herbicide damage, especially when it concerns the pigment and pigment precursors in photosynthetic apparatus.

Conclusions

The treatment of the oat plants with ALA, GA, and DP did not change the dynamics of the water exchange. The irradiation of the vegetable marrow plants treated with

ALA+DP and GA+DP caused a photodynamic effect, shown as a dehydration of the tissues and a decrease in the water potential. In treated plants, this induced an increased water uptake of the leaves from a solution with a low osmotic potential, due to formation of osmotic compounds during the oxidative stress. Those plants outflow the lowest amount of water in solutions with the highest osmotic potential, the reason being more in the higher water deficit, than in the elevated water keeping ability. Those changes are due to the cell damage from the oxidative stress causing an oxidation of plants membranes, leading to a dehydration of the treated plants. The photodynamic effect is a result of the abnormal accumulation of porphyrines, caused by the exogenous chlorophyll precursors ALA and GA. The way of action of early chlorophyll precursors ALA and GA is similar, which can be of some practical interests in designing new photodynamic herbicides.

References

Anderson J. V., J. L. Hess, B. J. Cheione, 1990. Purification, characterization, and immunological properties for two isoforms of glutathione reductase from eastern while pine needles. Plant Physiol., 94, 1402–1409.

Averina, N. G., N. V. Shalygo, E. B. Yaronskaya, 1989. Effect of glutamic acid and 1,10-phenantroline on the accumulation of chlorophyll precursors in green *Phaseolus* leaves. Photosynthetica, 23, 383–385.

Beale S. I., J. D. Weinstein, 1991. Biosynthesis of 5-aminolevulinic acid in phototrophic organisms. In: Chlorophylls. Ed. H. Scheer, CRC Uniscience series, Boca Raton, FL: 93–105.

Becana M., Moran J. and Iturbe-Ormaetxe I., 1998. Iron-dependent oxygen free radical generation in plants subjected to environmental stress; toxicity and antioxidant protection. Plant and Soil, 201, 137–147.

Becerril J. M., M. V. Duke, U. B. Nandihalli, H. Matsumoto, S. O. Duke, 1992. Light control of porphyrin accumulation in acifluorfen-methil-treated *Lemna pausicostata.* Physiol. Plant., 86, 6–16.

Becerril J. M., S. O. Duke, 1989. Protoporphyrin IX cotent correlates with activity of photobleaching herbicides. Plant Physiol. 90, 1175–1181.

Brey E. A., 2001. Plant response to water-deficit stress. Encyclopedia of life sciences. 1–5.

Bohner H. J., R. G. Jensen, 1996. Strategies for engeneering water-stress tolerance in plants. Trends in Biotechnology, 14, 89–97.

Chakraborty N., B. C. Tripathy, 1992. Involvement of singlet oxygen in 5-aminolevulinic acid-induced photodynamic damage of cucumber (*Cucumis sativus L.*) chloroplasts. Plant Physiol., 98, 7–11.

Granick S., 1961. Magnesium protoporphyrin monoester and protoporphyrin monoester in chlorophyll biosynthesis. J. Biol. Chem., 236, 1168–1174.

Gusev, N, 1960. Some methods of investigation of the plant water exchange. Acad. of USSR Publ., Moscow.

Knox J. P., A. D. Dodge, 1985. Singlet oxygen and plants. Photochemistry, 24, 889–896.

Koleva A., V. Toneva, 1998. Compared investigation of the photodynamic effect of 5-aminolevulinic acid, glutamic acid and 2,2´-dipyridil on oat and cucumber. Scientific works, University of Plovdiv, Plantarum, 34, 6, 152–162.

Koleva, A., V. Toneva, I. Minkov, 1995. Photodynamic effect of 2, 2'-dipyridyl and glutamic acid on wheat and vegetable marrow plants. Photosynthetica, 31, 189–196.

Mayfield S. P., T. Nelson, W. C. Taylor, 1986. The fate of chloroplast protein during photooxidation in carotenoid-deficient maize leaves. Plant Physiol., 82, 760–764.

Meier C. E., R. J. Newton, J. D. Puryear, S. Sen, 1992. Physiological responses of loblolly pine (*Pinus taeda* L.) seedlings to drought stress: osmotic adjustment and tissue elasticity. J. Plant Physiol., 140, 754–760.

Navari-Izzo F., C. Pinzino, M. F. QuartacciC. L. M. Sgherri, R. Izzo, 1994. Intracellular membranes: kinetics of superoxide production and changes in thylakoids of resurrection plants upon dehydration and rehydration. Proc. R. Soc. Edinburgh, 102b, 187–191.

Pelah D., O. Shoseyov, A. Altman, D. Bartels, 1997 a. Water-stress response in aspen (*Populus tremula*): differential accumulation of dehydrin, sucrose synthase, GAPDH homologues, and soluble sugars. J. Plant Physiol., 151, 96–100.

Pelah D., W. Wang, A. Altman, O. Shoseyov, , D. Bartels, 1997 b. Differential accumulation of water stress-related proteins, sucrose synthase and soluble sugars in *Populus* species that differ in their water stress response. Physiol. Plant., 99, 153–159.

Rebeiz C. A., A. Montazer-Zouhoor, H. J. Hopen, S. M. Wu, 1984. Photodynamic herbicides. I. Concept and phenomenology. Enzyme Microbiol.Technol., 6, 390–401.

Rebeiz C. A., A. Montazer-Zouhoor, J. M. Mayasich, B. C. Tripathy, S. M. Wu, C. C. Rebeiz. 1987. Photodynamic herbicides and chlorophyll biosynthesis modulators. In: Light Activated Pesticides, Vol. 339, Eds. J. R. Heitz, K. R. Downum, ACS Symposium Series, Washington, DC, 295–328.

Rebeiz C. A., J. A. Juvik, C. C. Rebeiz, 1988. Porphyric insecticides. 1. Concept and phenomenology. Pestic. Bochem. Physiol., 30: 11–27.

Smirnoff N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol., 125, 27–58.

Toneva V., A. Koleva, I. Minkov, 1997. Influence of glutamic acid and 2,2'-dipyridyl on the protochlorophyllide and protoporphyrin IX accumulation and their photodynamic action in green monocotyledons and dicotyledons. J. Plant Physiol., 150, 57–62.

Toneva V., I. Minkov, 2001. Influence of the photodestructive processes on the cell premeability in monocotyledonous and dicotyledonous plants, treated by early chlorophyll precursors and modulator of chlorophyll synthesis. Trav. Sci. Univ. Plovdiv., Plantarum, 37, 71–79.