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*Full Length Research Paper*

# Response of photosystem II and photosyntheticpigments to salt and Baikal EM1 in tree seedlings

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**The aim of the present research is to determine the effect of Baikal-EM1 preparation on photosystem II (PS II) activity and the synthesis of chlorophylls by tree seedlings in conditions of salt stress. This work reports, for the first time, the investigation of the influence of a preparation of effective microorganisms on the activity of PS II and the chlorophylls in the leaves of *Gleditschia triacanthos* L. and *Abies nordmanniana* Karst in conditions of salt stress. The methods used for the analyses of the structural components of these species allow the estimation of the condition of PS II and chlorophylls in normal plants and those that are subjected to salt stress. The analytical methods can also be used for diagnosing the resistance of plants to the action of salts. The results showed that salt stress appears to inhibit the activity of PS II and the synthesis of chlorophylls. The use of Baikal EM1 in both kinds of tree seedlings reduced the inhibiting effects of NaCl. The results obtained are a positive contribution to the effort to improve the salt resistance of seedlings, in that they increased our understanding of plants' resistance to the action of salts and identified an approach that increased the adaptive potential of the plants.**

**Keywords:** Tree, physiology, Baikal EM1, pigments, salt.

## INTRODUCTION

Plant production is limited by environmental stress. Christiansen (1982) pointed out the conclusion of Dudal (1976) that only about 10% of the world's arable land may be classified in a non-stress category. However, about 20% of the land is limited by mineral stress, 26% by drought stress and 15% by freezing stress (Blum, 1986). Most plants grow in environmental conditions that are, to a considerable degree, unfavorable to their growth. Each environmental factor usually has a minimum and maximum level, beyond which plants cannot survive (Howarth, 1991). Due to their stationary status, plants can only make metabolic and structural adjustment to cope with biological and non-biological trauma (Basra, 1994).

Environmental stresses are mainly of two types: Biotic (pathological organisms) and abiotic (physicochemical factors). Munns and Termaat (1986) and (Munns, 1993 stated that much of the physiological research into Salinity has concentrated on three topics: Water relations, photosynthesis and the accumulation of a particular metabolite, assuming that one or more of these processes would limit growth in saline soils. One of such processes, affected by environmental conditions, is photosynthesis. The tolerance of plants for unfavorable environmental factors depends on the adaptive ability of their photosynthetic apparatus (Strogonov, 1962; Ball and Anderson, 1986). Research has shown that the action of salt leads to the reduction of chlorophyll in leaves and decreases in the activity of photosystem II (PS II). PS II functioning represents the most sensitive indicator of environmental stress in plants, in that it comprised about 20 polypep-

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**Abbreviations: PS II,** Photosystem II; **EM,** effective microorganisms; **DCPIP,** dichlorophenol-indo-phenol natrium salt; **Chl,** chlorophyll.

tides that range in size from 4 to 50 kDa, 30 - 50 different chlorophyll molecules and several carotenoids. The PS II complex exceeds 300,000 molecular mass (Vermaas, 1995).

The role of pigments in plant processes is indispensable, whereas the synthesis of photosynthetic pigments is genetically controlled, but it also depends on environmental factors. It is known that the pigments presented in thylakoid membranes consist largely of two kinds of green chlorophylls: chlorophyll a and chlorophyll b. Also present are yellow-to-orange pigments classified as carotenoids. However, PS II contains chlorophyll a, β carotene (connected to two major proteins) and a small amount of chlorophyll b. These green bands represent light-harvesting complexes of pigments and protein, one of which functions with PS I and the other functions mainly with PS II. Their functions are to absorb light energy and transfer it to the proper photosystem, where it eventually reaches P700 or P680 (Cogdell, 1983).

Morgan and Austin (1986) showed that, for wheat, high rates of light-saturated photosynthesis are associated with the density of PS II reaction, which centers on the thylakoid membranes. This characteristic is associated with the low chlorophyll 73 content, a high chlorophyll a: b ratio and high rates of electron transport through PS II. Therefore, up to a certain extent, measurements of photosynthetic activity and chlorophyll concentration might give some idea about the tolerance that plant species have for a given stress condition.

In an earlier study, the action of salt stress and the synthetic hormone polystimuline K on the photosynthetic activity of *Trianea bogotensis* Karst was investigated and it was recommended that measurements of chlorophyll fluorescence *in vivo* should be used for rapid non destruction estimation of the salt tolerance of plant species (Allahverdiev, 1988).

An important component of organic agriculture is effective microorganisms (EM) technology and it is recognized as such in many countries. In 1988 in Japan (Higa Teruo) and 1997 in Russia (Shablin, 2006), the preparation of effective microorganisms was achieved using EM86 technology. In Russia, this preparation was given the name Baikal EM1. Effective microorganisms make amino acids useful to plants, and organic acids, polysaccharides and vitamins strengthen their immune systems. Baikal EM1 consists of a water solution that contains compounds that promote nitrogen fixation and photosynthesis, along with lactic bacteria, yeast and other components that these microorganisms need to live (Shablin, 2006).

The aim of the present research is to determine the effect of Baikal EM1 on PS II activity and on the synthesis of chlorophylls by tree seedlings in conditions of salt stress.

## MATERIALS AND METHODS

Two tree seedlings, that is, *Gleditschia triacanthos* L. (Gt) and *Abies nordmanniana* Y. (An), were used in this study. The seeds were surface sterilized by shaking for 20 min in a solution that contained 40% sodium hypochloride; then, they were rinsed thoroughly with sterilized, distilled water to remove the remaining sodium hypochloride. They were incubated in the dark at 22°C in an aqueous solution of Baikal EM1 (10 ml in 10 l of water, that is, 1:1000) for 18 h. The control seeds were incubated in distilled water for the same period of time and all of the seeds were then planted in plastic tumblers. The growth temperature was 25°C and the photo-cycle was 16 h light followed by eight hours of darkness. On the sixtieth day of growth, stress treatment begun, using 100 and 150 mM NaCl solutions. Seedlings that were 65 days old and exposed to salt stress for the last five days of that period were analyzed.

PS II activity measurements were conducted on the thylakoid membranes of the seedlings, while the thylakoid membranes were isolated from the control and treated plants. The leaf tissue was cut into small pieces and 500 mg were homogenized in 3 ml of a chilled, grinding medium (Appendix), using a Sorvall Omni-mixer at full speed for 15 seconds. The homogenate was filtered twice through four layers of cheesecloth. The filtrate was transferred to cooled centrifuge tubes and centrifuged at 3500 rpm for six minutes at 40°C by a Sorvall SS-34 rotor centrifuge. After centrifugation, the supernatant was discarded and the remaining pellet was dissolved in 4 ml of chilled suspension 119 medium (Appendix). The suspension was recentrifuged at 9000 rpm for 10 min at 40°C by a Sorvall SS-34 rotor centrifuge. The resulting pellet was suspended in 150 µl of suspension medium and stored in the dark on ice. PS II activities of the isolated thylakoid membranes were determined according to Chetti (1987). Equal amounts of thylakoid membrane isolates based on the chlorophyll concentration were assayed using dichlorophenol-indo-phenol natrium salt (DCPIP) as an electron acceptor. Thylakoid membranes containing 20 µg of chlorophyll were added to 2 ml of assay mixture (Appendix). The reaction was started by an illumination of the assay mixture with a lamp (Cole Parmer Instruments Co., Model 9741-52), and the activity measurements were conducted at 590 nm by a Shimadzu UV/VIZ spectrophotometer at 25°C. The absorbances, recorded by the spectrophotometer at 590 nm, were recorded every 15 s for a total of 180 s. The slopes of the curves were used as a measure of PS II activity. However, percent activities for both control and treated samples were calculated as

PS II-treated /PS II-control) x 100

Leaves from sixty days tree seedlings were grown in Evans and Nason (1953) nutrient medium (22°C, 80% humidity) for 12 h in the presence and absence of a Baikal EM1 solution (1:1000) and then placed in a salt solution (100 and 155 mM NaCl) for different periods of time (30, 60, 360 and 720 min). The chlorophyll (Chl) content of the leaves was determined (2% homogenate in 100% acetone) by a spectrophotometer at wavelengths of 645 and 663 nm (Shlyk, 1975).

## RESULTS AND DISCUSSION

The PS II activity of *G. triacanthos* and *A. nordmanniana* depended on the concentration of the salt (100 or 150 mM) and the treatment with Baikal EM1, as indicated in Table 1. Comparisons of control variants (without treatment by a preparation and with treatment) show that, in the case of treatment with Baikal EM1, the activity of PS II in *G. triacanthos* increases by 43.3%, while in *A. nordmanniana*, the increase was 40%. To make a

**Table 1.** PS II activities of *G. triacanthos* and *A. nordmanniana* in salt stress upon treatment with the Baikal EM1 preparation.

| Variant | *G. triacanthos* (Slope ± SD) | *A. nordmanniana* (Slope ± SD) |
|---|---|---|
| Control | 30±6 | 27±5 |
| Control + «BAIKAL-EM1» | 43±6 | 38±3 |
| 100 mm NaCl | 24±5 | 20±1.9 |
| 155 mm NACl | 20±3 | 15±5 |
| 100 mm NaCl + «BAIKAL-EM1» | 28±5 | 23±5 |
| 155 mm NaCl + «BAIKAL-EM1» | 23±2 | 16±1 |

**Table 2.** Changes of Chl a/b ratio in leaves of *G. triacanthos* grown in the absence (-) and presence (+) of Baikal EM1 preparation as a result of salt stress for various periods of time.

| Stress period (min) | Chl a/b ratio | | | |
|---|---|---|---|---|
| | 100 mm NaCl (-) | 100 mm NaCl (+) | 155 mm NaCl (-) | 155 mm NaCl (+) |
| 0 | 1.35 | 1.26 | 1.50 | 1.44 |
| 30 | 2.20 | 1.64 | 2.83 | 1.62 |
| 60 | 2.37 | 1.50 | 2.68 | 1.81 |
| 360 | 2.42 | 1.73 | 2.50 | 1.84 |
| 720 | 2.30 | 1.66 | 2.50 | 1.80 |

comparison between the plant species, the PS II activities of salt-treated plants were calculated as the percentage of the activities of control plants.

Compared to the control seedlings, there was a decrease both in *G. triacanthos* and *A. nordmanniana* under NaCl stress for the test seedlings. For the case of 100 mM NaCl, decreases in PS II activity of 20 and 26% were observed in *G. triacanthos* and *A. nordmanniana*, respectively. For the 155 mM NaCl stress condition, the decreases were 33.4 and 45.5% for *G. triacanthos* and *A. nordmanniana*, respectively. To check the protective effect of the Baikal EM1 preparation, similar experiments were performed under salt stress. For this purpose, the seeds were first treated with the preparation as in the case of the controls, while for the case of 100 mM NaCl, when compared with the control (without preparation), a decrease in PS II activity was observed in both species (Table 1).

However, after being treated with the preparation and upon being subjected to 100 mM NaCl stress, there was a 6.7% decrease in the PS II activity for the *G. triacanthos* seeds. For *A. nordmanniana*, at the same conditions, the PS II activity decreased by 15.0%, while for the salt stress condition of 150 mM NaCl, the PS II activity of *G. triacanthos* and *A. nordmanniana* decreased by 33.4 and 45.5%, respectively, when compared to the control (without preparation). When the Baikal EM1 treatment was combined with a stress condition of 150 mM NaCl, the PS II activity of *G. triacanthos* and *A. nordmanniana* decreased by 23.4 and 40.8%, respectively. To conclude, *G. triacanthos* has been determined to have a greater capacity for maintaining PS II activity under salt stress than *A. nordmanniana* does; thus, *G. triacanthos* can be referred as tolerant to salt stress on the basis of PS II activity.

Along with many physiological processes, photosynthesis is affected significantly by salt stress, as manifested by changes in chlorophyll molecules. Changes in chlorophyll content, in response to salt treatment, are evident in Tables 2 and 3. In, as little as, 30 min of salt stress conditions, both *G. triacanthos* and *A. nordmanniana* displayed increased Chl a/b ratios in the absence of the Baikal EM1 preparation, which can also be interpreted as a quantitative reduction of Chl b molecules (that is, biological breakdown) in PS II.

It is necessary to note that the greater Chl a/b ratio occurs at the higher concentration of salt (155 mM). In addition, the ratio of Chl a/b in the leaves of *A. nordmanniana* for both salt stress conditions is greater than the ratio in the leaves of *G. triacanthos*. However, in the presence of the Baikal EM1 preparation, such a reduction in the amount of Chl b was not observed, and the ratios fluctuated within limits that were close to those of the control. In light of the damage that occurs to Chl b molecules in PS II during salt stress, it is supposed that the main energy contribution to the plant, (in the form of ATP) is supplied from the more intensive work of the non-cyclic electron flow from PS I. *G. triacanthos* has been determined to have a higher capacity of maintaining PS II activity during periods of salt stress than *A. nordmanniana*; hence, it can be referred as tolerant to salt stress on the basis of PS II activity. The results of this research

**Table 3.** Changes in Chl a/b ratio in leaves of *A. nordmanniana* grown in the absence (-) and presence (+) of Baikal EM1 preparation as a result of salt stress for various periods of time.

| Stress Period (min) | Chl a/b ratio | | | |
|---|---|---|---|---|
| | 100 mm NaCl (-) | 100 mm NaCl (+) | 155 mm NaCl (-) | 155 mm NaCl (+) |
| 0 | 1.64 | 1.60 | 1.87 | 1.85 |
| 30 | 2.56 | 1.83 | 2.90 | 2.06 |
| 60 | 2.44 | 1.71 | 2.80 | 2.15 |
| 360 | 2.60 | 1.77 | 2.75 | 2.28 |
| 720 | 2.58 | 1.80 | 2.82 | 2.00 |

indicate that the Baikal EM1 preparation stimulates physiological processes in the investigated species of plants that reduce damaging effects of NaCl on PS II and the synthesis of chlorophylls. Results are further discussed in order to clarify how the different tolerance strategies produce such responses to salt.

## Conclusions

The results showed clearly that NaCl in both kinds of tree seedlings (*G. triacanthos* L. and *A. nordmanniana* Y.) inhibits the activity of PS II and the synthesis of chlorophylls. The greatest effect was noted for *A. nordmanniana*. The ratio of chlorophyll a to chlorophyll b varies according to the type of plant and as a result of salt stress. However, chlorophyll b is affected to a greater extent (greater inhibition). This could possibly be attributed to the chlorophyll damage at the PS II donor site, resulting in a decrease of Chl b molecules, which would increase the Chl a/b ratio. This research has clearly demonstrated the positive effect of the Baikal EM1 preparation on PS II activity and the synthesis of chlorophylls in tree seedlings. It seems likely that, in conditions of salt stress, Baikal EM1 stimulated physiological processes in the investigated plants. For the two plant species studied (*G. triacanthos* and *A. nordmanniana*), the former showed the greater stability when subjected to salt stress, based on the observed levels of its PS II activity and chlorophyll synthesis.

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