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Surhay Ragimođlu ALLAHVERDIEV

Meltem MAVİTUNA

Rena GANİEVA

Saeedeh NAFISI

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## Effects of Salt Stress and Synthetic Hormone Polystimuline K on Photosynthetic Activity of *Trianea bogotensis* Karst

Surhay Ragimoğlu ALLAHVERDIEV

Zonguldak Karaelmas University, Faculty of Forestry, Bartın-TURKEY

Meltem MAVITUNA

Department of Biology, Middle East Technical University, Ankara-TURKEY

Rena GANIEVA

Institute of Botany Academician Science of Azerbaijan Republic, Baku-AZERBAIJAN

Saeedeh NAFISI

Zonguldak Karaelmas University, Faculty of Forestry, Bartın-TURKEY

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**Abstract:** In the present study the effect of kinetin like synthetic hormone Polystimuline K (PS-K) on photosynthetic activity of salt stressed *Trianea bogotensis* Karst (*Hydrocharitaceae*) has been shown. Chlorophyll variable fluorescence (Fv) and slow fluorescence (SF) have been used to investigate the sensitivity of Photosystem II (PS II) to different salt stress treatments (103, 155 and 344 mM), at different time courses (15, 30, 60, 360 and 720 minutes). Salt stress (155 mM NaCl) treatments of the seedlings inhibited the SF magnitude approximately to 50 %. Pretreatment of seedlings for 30 minutes in 20 mg/l PS-K followed by salt stress resulted in an increase in SF magnitude. Under salt stress the magnitude of Fv/Fo decreases within all time measurements. In the presence of NaCl and PS-K until 60 min. of treatment there was a decrease in Fv/Fo value, then a slight increase was observed. The results of this study are important in understanding the protective function of PS-K on salt stress mechanism.

**Key Words:** *Trianea bogotensis* Karst, salt, polystimulin K, chlorophyll fluorescence

### Tuz Stresi ve Polistimulin K Adlı Sentetik Hormon'un *Trianea bogotensis* Karst'ın Fotosentetik Aktivitesi Üzerine Etkileri

**Özet:** Bu çalışmada kinetin benzeri sentetik hormon olan Polistimulin K (PS-K)'nin tuz stresi uygulanmış *Trianea bogotensis* Karst'ın (*Hydrocharitaceae*) fotosentetik aktivitesi üzerine etkisi gösterildi. Değişik zaman sürelerinde (15, 30, 60, 360 ve 720 dakika) farklı tuz stresi (103, 155 ve 344 mM) uygulamaları sonucunda, Fotosistem II (FS II)'nin hassasiyetini incelemek için Klorofil değişken Floresans (Chlorophyll variable fluorescence) (Fv) ve yavaş floresans (Slow Fluorescence) (SF) kullanıldı. Fideler üzerindeki tuz (155 mM NaCl) uygulaması SF boyutunu yaklaşık % 50 engelledi. Fidelerin 30 dakika süreyle 20 mg/l PS-K uygulamasına tabi tutulması ve daha sonra tuz ortamına alınması sonucunda SF boyutunun yükselmesi saptanmıştır. Tuz stresi altında, bütün zaman süreçlerinde, Fv/Fo oranı azalma göstermektedir. Uygulamaların 60 dakika'sına kadar NaCl ve PS-K'nin bulunduğu ortamda Fv/Fo miktarı bir düşüş gösterip, daha sonra hafif bir yükseliş tespit edildi. Bu çalışmanın sonuçları PS-K'nin tuz stresi mekanizmasındaki koruyucu görevini anlamak açısından önemlidir.

**Anahtar Sözcükler:** *Trianea bogotensis* Karst, tuz, polistimulin K, klorofil, floresans

### Introduction

The environmental stress has been the main problem of the plants which significantly effect the germination, development and reproductivity. The evolutionary adaptation of plants to arid environment was the result of combined effect of environmental factors such as low or high temperature, drought, high salt content of the soil, high or fluctuating ionic strength in the soil.

Attention is still focused on the unsolved problem of stress tolerance and adaptation mechanisms employed by plants at molecular level (1, 2). The structure and function of the photosynthetic apparatus of plants respond to changes in environmental conditions, especially those of salt, drought, heat and light stress.

Salt tolerance mechanism in higher plants are known in general terms. Among these at least two mechanisms storage and excretion explain such com-

plex physiological processes (3-8). For understanding the salt tolerance mechanism, photosynthetic activity measurements have also been used. Smillie and Nott (9) emphasized the advantages in using of chlorophyll fluorescence kinetic analysis in characterization of stress related effects in closely related species or varieties. The study of light-induced in vivo chlorophyll fluorescence of green plants provides basic information on the functioning of the photosynthetic apparatus (10) chlorophyll fluorescence measurements which have been used in assessing the effects of a variety of stresses on crop plants (11-13). Krishnaraj et al. (14) used the chlorophyll fluorescence measurements in screening of salt tolerant wheat varieties. It has large applications in selection of heat and cold tolerant plant species. Various stress conditions may reduce the rate of photosynthesis, and disturb the light-driven photosynthetic electron transport via heat emission and chlorophyll fluorescence. This inverse relationship between in vivo chlorophyll fluorescence and photosynthetic activity can be used to study the potential photosynthetic activity of leaves and to detect stress effects on green plants (15). There are several parameters of the in vivo chlorophyll fluorescence which can be applied for detecting stress and damages of the photosynthetic apparatus. Variable fluorescence ( $F_v$ ) and initial fluorescence ( $F_o$ ) ratio is one of these parameters and it shows the efficiency (16). Data relating to the effect of salinity on the primary photochemical reactions under in vivo conditions, however are limited and conflicting.

In the present work the physiological response of plant species *Trianea bogotensis* Karst to salt stress and the contributions of the plant hormone regular PS-K on photosynthetic activity were investigated. Salt stress induced changes in photosynthetic activity and effect of PS-K was followed by chlorophyll fluorescence measurements.

## Materials and Methods

### Plant Material

In this study, *Trianea bogotensis* Karst, which is a highest water (lake) flowering plant species, is used. Young seedlings (7-days) were grown in Hoagland Arnon (17) nutrition solution for 40 days in a growth chamber on 16 hours light and 8 hours dark period at  $25^{\circ}\text{C} \pm 2,80\%$  humidity.

### Salt Stress and PS-K Treatments

The salt stress treatments on 47 days old seedlings were achieved in the same solution as 103 mM, 155 mM and 344 mM NaCl. Polystimulines (PS) are a new group of synthetic substances capable of increasing adaptive potential of plants and definite extent to improve their metabolic activity in stress conditions (18). Being the analogs of cytokinins (Polystimulin K or PS-K) it is classified among synthetic phytoactive polymers. The PS-K (20 mg/l) was also included in the same nutrient solution.

### Chl Fluorescence Measurements

Fluorescence measurements were made according to the procedure of Peeler and Naylor (19). Induced chlorophyll fluorescence was measured over a 10 sec excitation period by the help of PAM-101 Chlorophyll Fluorometer (H. Walz, Germany). All the leaf segments (four to six) excited from plants were placed on moistened 3 mm filter paper sitting on a wet sponge in a glass dish and covered with transparent plastic film. The measurements were taken at  $25^{\circ}\text{C}$  directly placing the probe on the surface of the leaves. The kinetics of the SF were measured using phosphoroscope during the time between the measurement of fluorescence which was equal to 1.25 seconds. Photocurrent registration was made using a direct current amplifier with output sensitivity of 100 Mohm and chart recorder KSP-4 with an integrator I-02 which permitted to determine the area under the SF curve. S $F_o$ -p phase reflects the state of chemical component of electrochemical proton gradient. S $F_p$ -s phase characterizes electron transport velocity.  $F_v$  reflects the functional photosynthetic electron transfer chain and attended processes.  $F_o$  means initial fluorescence. The fluorescence indices  $F_o$ ,  $F_v$  and  $F_v/F_o$  are automatically calculated and displayed. Biological repetition of the experiments was carried out three times for each variant, and measurements were made on 4-6 standard cuts of experimental plant leaves.

## Results and Discussion

In the present work the plant species *Trianea bogotensis* Karst is stressed with different NaCl concentrations at different time periods. The experiments were repeated in the presence of synthetic hormone PS-K and effect of salt stress was followed by photosynthetic activity measurements.

In Figure 1, effect of 155 mM NaCl and PS-K (20 mg/l) on the fluorescence induction curves of *Trianea bogotensis* leaves were shown. Treatment of plants with 155 mM NaCl for 30 min. exhibited 50 % decrease in SF. However, treatment of plants within the

presence of synthetic hormone PS-K, salt stress induced inhibition was partially removed.

SFo-p values were measured at different salt concentrations (103, 155 and 344 mM NaCl) at different time periods (15, 30, 60, 360 and 720 min.) and the results are given in Figure 2.

As salt concentration and stress duration time increase, SFo-p values decrease and maximum inhibition was observed at 344 mM NaCl concentration. SFo-p values are higher in the presence of PS-K in salt variants (105,155 mM) comparing to those when PS-K is not applied (Figures 2; a, b). The protective effect of PS-K on Photosynthetic activity of salt stressed plants was 103 mM in 60 min. and 155 mM in 30 min.

In Figure 3 the relative changes of SFp-s values under different salt stress and at different stress durations are given. Again the protective effect of PS-K was reveal in 103 and 155 mM NaCl treatments. However, in hypersalt concentration (344 mM), the PS-K does not influence the SFo-p and SFp-s values (Figures 2 (c) and 3 (c)).

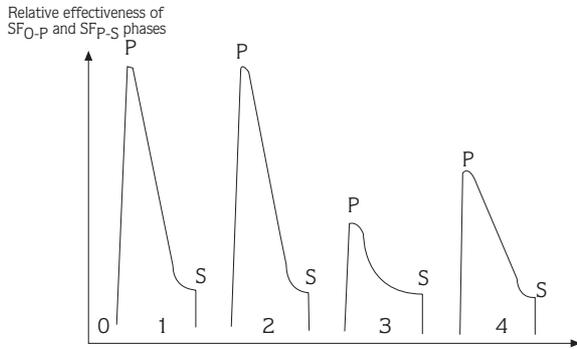


Figure 1. Inductive curves (SF) of *Trianea bogotensis* leaves under salt and PS-K treatments. 1: Control; 2: PS-K-30 min. (20 mg/l); 3: NaCl (155 mM)-30 min.; 4: NaCl (155 mM) + PS-K-30 min. (20 mg/l).

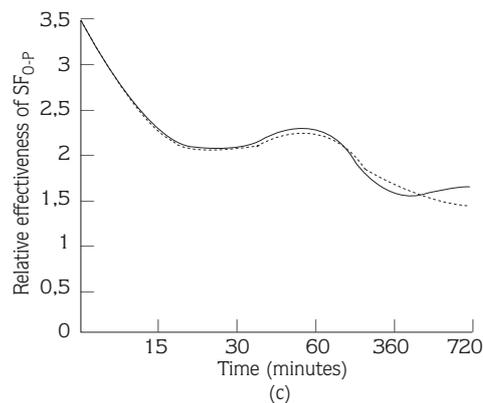
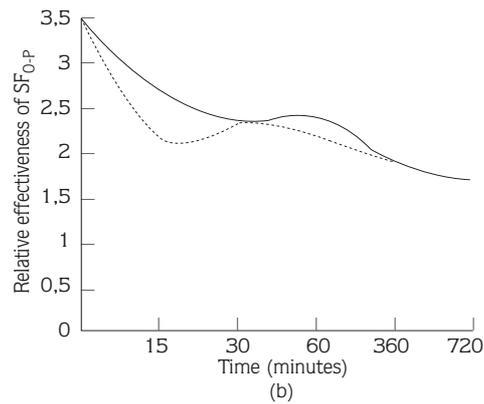
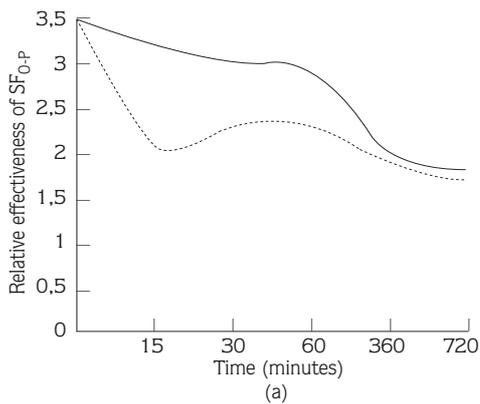


Figure 2. Changes of character of SF<sub>o-p</sub> curves under salt stress conditions and PS-K. a) 103 mM NaCl; b) 155 mM NaCl; c) 344 mM NaCl. — : NaCl+PS-K (20 mg/l); - - - - - : NaCl

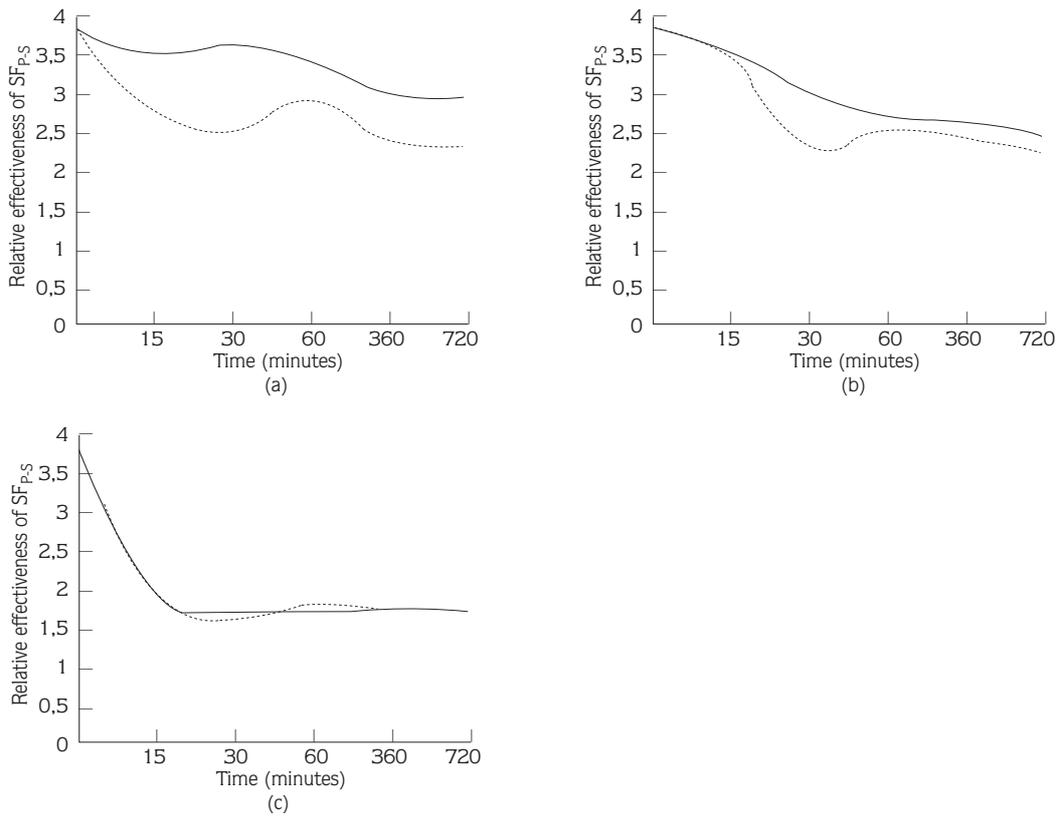


Figure 3. Changes of character of  $SF_{P,S}$  curves under salt stress conditions and PS-K. a) 103 mM NaCl; b) 155 mM NaCl; c) 344 mM NaCl  
 — : NaCl+PS-K (20 mg/l); - - - : NaCl

Effect of salt stress on PS II was also monitored by chlorophyll fluorescence measurements. The measurements were taken on control, salt treated, PS-K treated, salt and PS-K treated samples at different time periods. The result were shown in Figure 4 as the ratio of the  $F_v$  over the initial fluorescence ( $F_o$ ). As it is clear from the figure, under salt stress the magnitude of  $F_v/F_o$  decreases within all time measurements. PS-K itself also exhibited similar type of reduction in 60 min. while in the presence of salt and PS-K in the same period of time of treatment there was a decrease in  $F_v/F_o$  value then a slight increase was observed. The ratio of  $F_v$  to  $F_o$ , that is used in this analysis, varies in relation to the changes of  $F_v$ ,  $F_o$ , or both  $F_v$  and  $F_o$ . Analysis of the transients obtained in the present study showed that changes in the  $F_v/F_o$  ratios were associated mainly with changes in  $F_v$ .

The results presented demonstrate the potential utility of measurements of chlorophyll fluorescence in vivo for rapid non-destructive estimation salt tolerant plant species.

The results of this study are important for both screening applications of chlorophyll fluorescence in se-

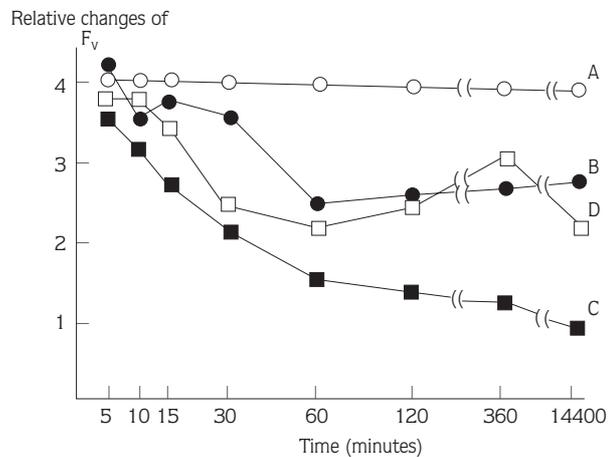


Figure 4. Changes of variable fluorescence (expressed as  $F_v/F_o$ ) time of *Trianea bogotensis* leaves under salt stress conditions and PS-K. A: Control; B: NaCl (344 mM) + PS-K (20 mg/l); C: NaCl (344 mM); D: PS-K (20 mg/l).

lection of salt tolerant plant species and the protective role of PS-K, it also provides information on the salt

tolerance mechanism at development stages including the photosynthetic electron transfer systems. PS-K has clearly expressed antistress properties. Its action leads to the correction of examined parameters, suppressed by salt stress. The inhibitory effects of NaCl in synthetic processes is high when it is applied alone, but it decreases in the presence of NaCl together with PS-K.

The knowledge in the literature and the data found from our studies show cytokinins in particular have both direct and indirect effects on functional activity

chloroplasts and their chlorophyll fluorescence (20). These results indicate that in *Trianea bogotensis* Karst subjected to severe salt stress the non-stomatal component of photosynthesis was affected and perhaps a light-dependent inactivation of the primary photochemistry associated with photosystem II (PS II) occurred.

Results are further discussed in order to clarify the different tolerance strategies underlying such responses to salt.

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