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**Researche Article**

# Effect of Salt Stress and Synthetic Hormone Polystimuline K on The Photosynthetic Activity of Cotton (*Gossypium hirsutum*)

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**Abstract:** The effects of Polystimuline K (PS-K), as a cytokinin analogue, on delayed light component state (DF), intermittent fluorescence (IF) and chlorophyll (Chl) a/b ratio was investigated in the leaves of different genotypes of cotton (*Gossypium hirsutum* L.) under salt stress (155 mM Na Cl) for different periods of time. It was shown that in the early perioıd of salt stress, DF and IF amplitudes are decreased, which is indicative of a Photosystem II (PS II) activity decrease. It is possibly related to the damage of chlorophyll in the PS II donor site and the decrease of Chl b molecules leading to an increased Chl a/b ratio. However, the improvement of the affected variables as a consequence of PS-K pretreatment suggests the stablizing effects of the synthetic growth regulator on the thylakoid membranes leading to a normalization of $H^+$/NaCl exchange that consequently hastens the recovery of damaged PS II centers.

Key Words: Polystimuline K, chlorophyll fluorescence, salt stress, photosystem II, cotton

## Tuz Stresi ve Polistimulin K Adlı Sentetik Hormonun Pamuk'un (*Gossypium hirsutum*) Fotosentetik Aktivitesi Üzerine Etkileri

**Özet:** Bir sitokinin anaoğlu olan Polistimülin'nin K (PS-K) farklı periotlarda tuz stresine (155 mM NaCl) maruz kalmış farklı pamuk (*Gossypium hirsutum* L.) genotuplerindeki gecikmiş ışık komponent durumu (DF), aralıklı flöresans (IF) ve klorofil (Chl a/b) oranı üzerindeki etkisi araştırıldı. Tuz stresinin erken aşamalarında, DF ve IF dalga boylarında düşmeye neden olduğu ve bunun fotosistem II (PS II) aktivitesinin azalması yoluyla ortaya çıktığı gösterilmiştir. Bu düşüş, alıcı PS II merkezindeki klorofil b moleküllerinin azalması ve bunun sonucu olarak Chl a/b oranının yükselmesiyle oluşmaktadır. Buna karşın, etkiye maruz kalan değişkenlerin PS-K ön-muamelesi sonucu düzelmesi, bu sentetik büyüme düzenleyicisinin tiylakoid membranları üzerinde kararlılık kazandırıcı etkisi olduğunu ortaya koymuştur. Bu kararlılık, öncelikle $H^+$/NaCl değişiminin normalize edilmesi ve böylece zarara uğramış PS II merkezinin onarımının hızlandırılmasına neden olmaktadır.

Anahtar Sözcükler: Polistimülin K, klorofil flöresans, tuz stresi, fotosistem II, pamuk

## Introduction

Salinity, as well as many other stress factors, is known to cause changes in physiological processes in plants (1). One such process affected by environmental conditions is photosynthesis. A plants' tolerance toward unfavorable environmental factors depends on the adaptive ability of the photosynthetic apparatus (2, 3).

Chl fluorescence induction (kinetics) reflects the photochemical activities of the photosynthetic apparatus. One of the integral indices of the status of the photosynthetic apparatus is delayed (DF) and intermittent (IF) fluorescence of chlorophyll molecules that are involved in the electron transport chain, energization of photosynthetic membranes and maintenance of reaction centers (4).

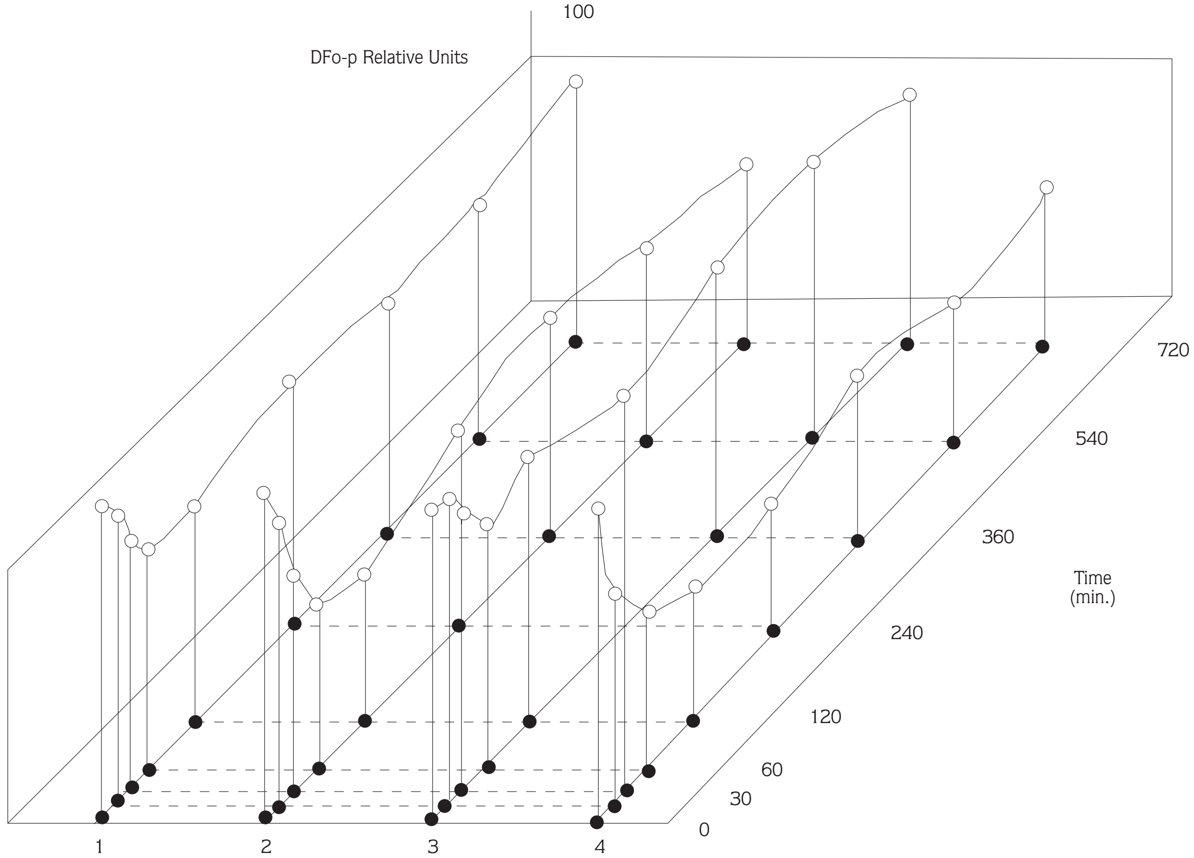


Figure 1.(≺) Dependence of DFo-p character changes on PS-K in cotton leaves of the two genotypes under stress (NaCl-0.9%) for various periods of time.
1. C-6524 variety in the presence of the PS-K
2. C-6524 variety without PS-K
3. Tashkent variety in the presence of the PS-K
4. Tashkent variety without PS-K

Phytohormones have an important role in the regulation of plant metabolism, coordinating the functional activites of whole plants as well as their reaction to a wide variety of stresses. Cytokinins in particular were observed to have both distant and direct effects on the functional activity of chloroplasts (5).

Like many other synthetic growth regulators, organic chemicals resembling natural cytokinins have been synthesized and tested for their cytokinin-like activities. PS-K is one such organic compound having cytokinin-like activities (6). The aim of this study was to investigate effects of this compound on the flourescence characteristics of chlorophyll in cotton leaves (*Gossypium hirsutum* L.) under salt stress in order to determine its future potential in agriculture.

## Materials and Methods

Leaves from two-week-old cotton seedlings ("Tashkent" and "C-6524" varities) were grown in Hoagland-Arnon (7) nutrient medium ($22^\circ$, 80% humidity) for 12 hrs. in the presence and absence of Polystimulin K (PS-K) (30 mg/l), then placed into salt solution (NaCl, 155mM) for different periods of time (30, 60, 120, 240, 360 and 720 min.).

Contents of Chl a and b were determined in the leaves (2% homohenate in 100% aceton) by spectrophotometry (SF-26) at wave lengths of 662 and 64 nm (8).

Delayed flourescence (DF) kinetics were measured by means of a phosphoroscope with the time between excitation and fluorescence equal to 1.25 sec. (9). Photoflow was registered by direct current amplifier with an output resistance of 100 Mohm on KSP-4 recorder with integrator (I-20 with digital printing device), allowing evaluation of the area under the DF peak. Induction of intermittent fluorescence (IF) was measured by a Pecordera-PAM-101 Chlorophyll Fluorimeter (H.

Table 1. Changes of Ch a/b ratio in leaves of cotton grown in the presencee (+) and absence (-) of PS-K under salt stress (155 mM NaCl) during various periods of time.

| Stress Period (minutes) | Chl a/b | | | |
|---|---|---|---|---|
| | C-6524 Variety | | Tashkent Variety | |
| | PS-K (+) | PS-K (-) | PS-K (+) | PS-K (-) |
| 0 | 2.64 | 2.43 | 2.56 | 2.50 |
| 30 | 2.81 | 4.94 | 2.85 | 3.87 |
| 60 | 3.17 | 4.90 | 2.96 | 3.72 |
| 360 | 3.09 | 4.86 | 2.93 | 3.70 |
| 720 | 2.90 | 4.87 | 2.79 | 3.72 |

Walz, Germany). The object was excited with white light for 10 seconds. The measurements were taken at 25°C with the probe placed directly on the surface of the leaves.

The DFo-p phase reflects the state of chemical proton gradient. The DFp-s phase characterizes electron transport velocity. Fv reflects the functional photosynthetical electron transfer chain and attendant processes. The symbol Fo refers to initial fluorescence. The fluorescence indices Fo, Fv and Fv/Fo 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

The DF induction curve records given in Figures 1 and 2 show that, under NaCl stress, the amplitude and steepness of ascent decreased and half-width of induction maximum of quack phase of DFo-p increased, reflecting the condition of the electrochemical gradients of the protons. The DFp-s phase characterizing the rate of electron transport also decreased. The presence of PS-K causes significant characteristic shifts of DP parameters induced by salt stress in both genotypes. This salt effect

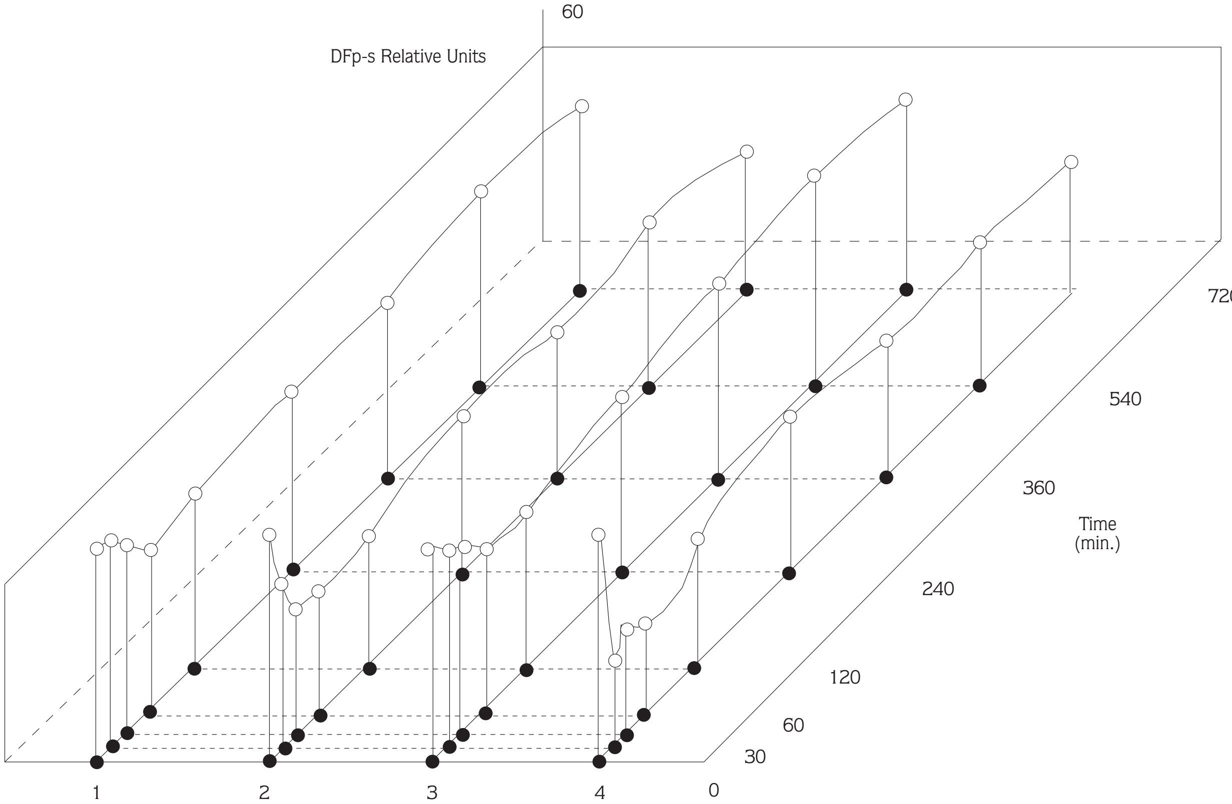


Figure 2. Dependence of DFp-s character changes on PS-K in cotton leaves of the two genotypes under salt stress (NaCl-0.9%) for various periods of time.
1. C-6524 variety at presence of the PS-K
2. C-6524 variety without PS-K
3. Tashkent variety at presence of the PS-K
4. Tashkent variety without PS-K

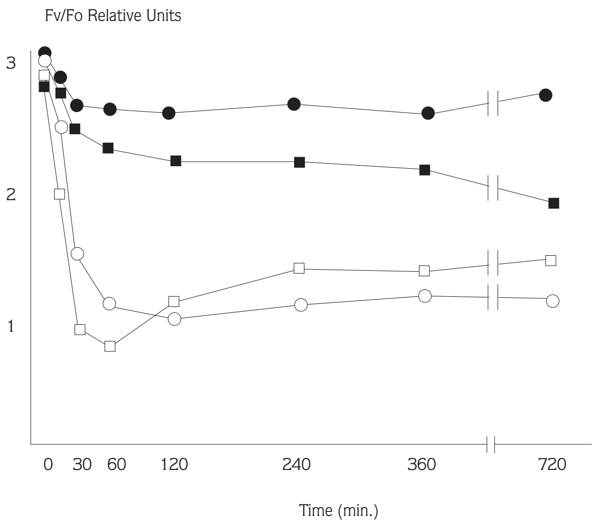


Figure 3. Fv/Fo ratio dependence of PS-K in cotton leaves of the two genotypes under salt stress (NaCl-0.9%) for various periods of time.
● C-6524 variety with PS-K
■ Tashkent variety with PS-K
□ Tashkent variety without PS-K
○ C-6524 variety without PS-K

is manifested most significantly during the short period under stress. When the stress period is increased, DFo-p phase intensity recovers to sme extent and stabilizes at a level still lower than that of the control (Fig.1) (≺). The same is also observed for DFo-p phase (Fig.2) (➢). However, we noted that the Tashkent value was equivalent to that of the C-6524 variety. The presence of PS-K sharply changes the effects of salt on both DF phases (DFo-p and DFp-s). A decrease in DF intensity was detected during the initial stress period but then leveled off and subsequently increased and stabilized at a level close to that of the control. The strongest PS-K effect was observed in the less stable variety Tashkent.

Rapid testing of the tolerance of plants to various strees factors is an important tool for plant physiologists. To be able to achieve this, various parameters can be used. The rate of the intermittent and background part of flourescence (Fv/Fo) (Fig.3) (⅄) can serve as a very informative parameter for salt stress.

In the absence of PS-K preatreatment, the Tashkent variety was observed to be most affected by short-term (upto 60 min.) salt stress, hence displaying dramatic Fv/Fo value reductions (Fig.3). Although PS-K pretreatment reverses this effect for both varieties, C-6584 was noted to be the superior variety inasmuch as it displayed very slight Fv/Fo value reductions and is therefore more resistant to salt stress.

Along with many physiological processes, photosynthesis is affected significantly by salt stress, as manifested by changes is chlorophyll molecules. When Table 1 is examined, changes in chlorophyll content as a response to salt treatment can be easily observed. In as little as 30 minutes under saline conditions, both varieties displayed increased Chl a/b ratios in the absence of PS-K, which can also be interpreted as a quantitative reduction of Chl b molecules (i. e., biological breakdown) in PS II. However, in the presence of PS-K, such a marked reduction in the amount of Chl b was not observed, the ratios fluctuating close to the control values. In light of the damage to Chl b molecules in PS II occuring during salt stress, we suggest that the main energy contribution, in the from of ATP, to the plant is supplied from the more intensive work of non-cyclic electron flow from Photosystem I.

Cytokinins are known to increase the tolerance of plant cells to various unfavourable conditions. Although the details of the protective nature of cytokinin action are not known, the general mechanisms of proposed are strikingly similar. For example, cytokinins act on the structural and functional states of different macromolecular cell components, in particular, on the state of their membranes. Cytokinins are known to slow granum damage in chloroplasts (10), to activate photosynthesis in leaves (11), to increase incorporation of labelled amino acids into proteins within chloroplasts *in planta* and in isolated chloroplasts (12), to prevent degradation of the chloroplast inner membrane apparatus typical of ageing in yellowing leaves (13, 14), to cause secondary grana formation (15), and generally to increase the chlorophyll and total protein content of chloroplasts (16).

The protective function of PS-K appears similar to the protective action of cytokinins (17, 18). It was shown, in particular, that treatment of winter wheat by PS-K increased its tolerance to frost from 30% to 50%. Electron microscopic examinations have revealed reconstruction of membrane systems in wheat chloroplasts which contained fewer grana but more thylakoids.

Changes in the electron tranport and electrochemical gradient of protons within membranes observed under salt stress during our investigations show an inhibiting effect of NaCl on these prameters. Sharp DF phase suppression during the first minutes of stress, particularly in the phase characterizing the efficient activity of the electron transfer chain, shows that the NaCl effect may be associated with damage of donor side of PS II by washing out peripheral proteins (19). Elimination of the salt effect by PS-K during the first minutes of stress may be the result of its stimulating the recovery and synthesis processes, which increase the adaptive abilities of the plant. We suggest that PS-K possibly switches on a mechanism which carries the damaged PS II center into the stroma lamella for turnover, while in the meantime a new PS II is established (synthesized), again by the involvement of PS-K.

Another model could also be postulated in which PS-K, by modulating the formation rate of $CO_2$-fixation reaction products, may hasten the recovery of damaged PS II centers, since these products (as well as cytokinin (PS-K) induced proteins) participate in charge interactions with the PS II centers.

With this investigation, we have clearly demonstrated the positive effects of PS-K on the salt tolerance of at least two varieties of cotton. However, to be able to generalize this effect to other plant species, more research should be conducted.

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