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Kinetin and spermine mediated induction of salt tolerance in wheat plants: Leaf area, photosynthesis and chloroplast ultrastructure of flag leaf at ear emergence

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ABSTRACT

The antagonistic effects of kinetin and spermine on stress imposed by seawater on leaf area, pigment, Hill reaction, $^{14}\text{CO}_2$ fixation and chloroplast ultrastructure of wheat flag leaf were investigated. Irrigation of wheat plants by seawater at 25% caused marked decrease in leaf area, pigment content, Hill activity and photosynthetic efficiency of wheat flag leaf at ear emergence. Grain priming with kinetin, spermine or their interaction alleviated the adverse effect of seawater stress by stimulating leaf area expansion, pigment production as well as photosynthetic activity. From transmission electron microscopy micrographs, a continuous “end-to-end” distribution of regular (oval or elliptical) chloroplasts around the cell’s periphery was observed in flag leaf mesophyll cells of control wheat plants. Conversely for seawater-stressed plants, the irregular spherical chloroplasts appeared “bulbous” and discrete, the cells also displayed extensive but thin peripheral cytoplasmic regions devoid of chloroplasts. Grain presoaking in 0.1 mM kinetin caused the chloroplast of stressed wheat plants to be more regular, with organized membrane system, large starch grains and projections in the form of tails. Furthermore, ultrastructure analysis cleared that grain priming with spermine, either alone or in combination with kinetin, caused the chloroplast in flag leaf mesophyll cells of stressed wheat plants to be more regular in shape with more starch grains. The changes in pigment content and photosynthetic activity of flag leaf appeared to depend mainly on chloroplast ultrastructure and its numbers, showing a positive correlation between chloroplasts number and pigment content.

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Abbreviations: K, kinetin; LSD, least significant difference; Spm, spermine; SW, seawater; (2, 6-DCPIP), 2, 6 dichlorophenolindophenol.

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1. Introduction

Salt stress is a global problem in crop production, and secondary salinization in soils of protected cultivation has become one of major factors adversely affecting horticultural crops. Salt stress affects every aspect of physiology in crops, as a result virtually reduces their productivity and adversely affects their product quality [1].

Photosynthesis is one of the primary processes most affected by abiotic stresses [2]. Additionally, plant pigments are known to serve a variety of purposes, and are thus critical to the function and health of plants, though the relative concentrations of these pigments can vary significantly depending not only on the species but also on the surrounding environmental factors [3]. It is well documented that salt stress exerts its deleterious effects on plant growth and development through its induction to the reduction in Chl a, Chl b, carotenoids and total pigments [4]. Aldesuquy and Gaber [5] found that seawater at 25% induced massive reduction in soluble, insoluble and total photosynthates as well as ratio of soluble/insoluble photosynthates during growth and development of *Vicia faba* plants. Furthermore, reduction in the pigment content by various stress conditions was previously recorded [6]. Clear reduction in Chl a, Chl b and carotenoids concentrations was observed in pineapple explants treated with seawater [7]. High salinity induced a decrease in photosynthetic efficiency is often associated with inhibition of photosystem II (PSII) [8,9]. PSII has been considered the main site of salt stress-mediated damage to electron transport processes [10], yet the damage site of PSII seems to vary with the severity and duration of stress [11] and plant species [12].

Electron microscopy showed that in salt-stressed plants, the structure of the chloroplasts becomes disorganized, the number and size of plastoglobuli increased and their starch content decreased [13]. Similarly, under saline conditions, the chloroplasts of *Aster tripolium* plants were partially horseshoe-shaped. Their thylakoid membranes showed dilations, the spaces between the membranes looked swollen and undilated thylakoid areas were developed. In particular, the number of grana stacks was considerably reduced [14].

Plant growth regulators (PGRs) have been found to play a key role in the integration of the responses expressed by plants under stress conditions [15]. Many reports have indicated that stress tolerance of plants is correlated with their capacity to enhance the synthesis of polyamines upon encountering the stress [16].

Salt stress, especially at the higher NaCl concentration, was found to induce a substantial decrease in leaf area and stomata conductance; chlorophyll content and δ -amino-levulinic acid dehydratase (ALA-D) activity were also affected, resulting in the lower net photosynthetic rate and dry matter production. Meanwhile, kinetin was found to reduce appreciably the adverse effects of salinity, besides favorably modulating antioxidant enzyme activities and alleviating oxidative stress in the test plants, to result in a higher yield as compared to the untreated stressed plants. Overall, the results indicate an optimization of antioxidant defense mechanisms and physiological processes by kinetin and a significant role of

exogenous phytohormones in conferring salt tolerance in *Nigella sativa* L. [17,18]. Shu et al. [1] concluded that Spermidine can alleviate salt-induced damage on cucumber seedlings by regulating the levels of endogenous polyamines, which was associated with an improvement in the photochemical efficiency of PSII of the salt stressed plants.

The objective of this study was to determine the effects of exogenous kinetin, spermine or their interaction on leaf area expansion, pigment content, photosynthetic activity and chloroplast ultrastructure of flag leaf as well as their role in alleviating the damage induced by seawater stress.

2. Materials and methods

2.1. Plant material and growth conditions

For soaking experiment, a homogenous lot of *Triticum aestivum* L. var. Sakha 93 grains were selected. The grains were surface sterilized by soaking in 0.01 M HgCl₂ solution for 3 min, then washed thoroughly with distilled water. The sterilized grains were divided into four sets. Grains of the 1st set were soaked in distilled water to serve as control, while those of the 2nd, 3rd or 4th set were soaked in 0.1 mM kinetin, 0.3 mM spermine or 0.1 mM kinetin + 0.3 mM spermine; respectively, each for about 12 h. After soaking, thoroughly washed grains were drilled in 15th November 2011 in plastic pots (20 cm in diameter) filled with 5.5 kg soil (clay/sand 2/1, v/v), where fifteen grains were sown in each pot. The pots were then kept in a greenhouse at Botany Department, Faculty of Science, Mansoura University, Egypt. The plants were subjected to natural day/night conditions (minimum/maximum air temperature and relative humidity were 15/25 °C and 35/45%; respectively) at mid-day during the experimental period. The plants in all sets were irrigated to field capacity by tap water. After two weeks from sowing, thinning was started so that five uniform seedlings were left in each pot for the subsequent studies. The plants of each set were sub-divided into two groups. The 1st group in each set was still irrigated with normal tap water serving as control, whereas the 2nd one was irrigated with 25% seawater (volume/volume). The resulting eight treatments were marked as following: 1. Control (Cont.), 2. Seawater (SW), 3. Control kinetin (Cont. K), 4. Seawater + kinetin (SW + K), 5. Control spermine (Cont. Spm), 6. Seawater + Spermine (SW + Spm), 7. Control kinetin + spermine (Cont. K + Spm), 8. Seawater + kinetin + spermine (SW + K + Spm). Irrigation with seawater was applied after 30 days from sowing with a periodical soil washing (each two weeks) with tap water. The chemical analyses of the employed seawater, collected from the Mediterranean Sea, revealed that it contains Cl⁻, 21.6 kg m⁻³; Na⁺, 11.1 kg m⁻³; SO₄²⁻, 2.85 kg m⁻³; K⁺, 0.49 kg m⁻³ and P³⁺ 16.6 μg dm⁻³. Its salinity was found to be 38.5 g kg⁻¹; pH, 8.1 and electric conductivity (EC), 47 mmhos cm⁻¹ [19]. After thinning and at heading, the plants received 36 kg N ha⁻¹ as urea and 25 kg P ha⁻¹ as superphosphate. Measurements were carried out at ear emergence (i.e. 65 d from planting). Samples from the flag leaf of the main shoot were taken as follow: ten replicates for

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