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# Kinetin alleviates the influence of waterlogging and salinity on growth and affects the production of plant growth regulators in *Vigna sinensis* and *Zea mays*

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**Abstract** – Growth criteria (shoot height, root length and dry weight) of 14-d-old *Vigna sinensis* and *Zea mays* were mostly suppressed by waterlogging or salinization using artificial seawater mixture during the subsequent 3 weeks; the water level in pots was, respectively, kept at 120% or 60% of water field capacity. The suppression in growth induced by salinization was greater than that obtained by waterlogging. The pattern of changes in growth appeared similar to chlorophyll a and b as well as activity of  $\delta$ -aminolevulinic acid dehydratase (ALA-D). On the other hand, waterlogging significantly increased indole-acetic acid (IAA) in shoots of both species but salinity had a decreasing effect. Both treatments decreased gibberellic acid (GA<sub>3</sub>) levels in shoots of *Vigna sinensis* and *Zea mays* as well as zeatin in shoots of *Zea mays*. Meanwhile, abscisic acid (ABA) was greatly accumulated in shoots of the stressed plants. Foliar application of 50 ppm kinetin counteracted the resulting reduction in growth and in chlorophylls of both species but partially lowered the inhibition in ALA-D activity. Moreover, kinetin increased IAA, GA<sub>3</sub> and zeatin in the stressed plants to mostly reach control levels, but markedly reduced ABA. These findings indicate that relief of the damage and restoration of normal conditions was maintained either partially or completely by application of kinetin. This recovery may be a consequence of several roles played by such hormones, which can cause triggering of the internal cellular metabolism and also induce alterations in the ratios of growth regulators.

plant growth regulators / salinity / *Vigna sinensis* / waterlogging / *Zea mays*

**Résumé** – La kinétine permet d'éviter l'effet de la submersion et de la salinité sur la croissance et affecte la production de régulateurs de croissance chez *Vigna Sinensis* et *Zea mays*. La croissance (hauteur des parties aériennes, longueur des racines et poids sec) de plantules de *Vigna sinensis* et *Zea mays* âgées de 14 jours a été arrêtée principalement par la submersion ou la salinisation en utilisant un mélange artificiel d'eau de mer durant les 3 semaines suivantes ; le niveau d'eau dans les pots a été maintenu respectivement à 120 % et 60 % de la capacité au champ. L'effet négatif sur la croissance induit par la salinisation était plus important que celui obtenu par la submersion. La forme des modifications de la croissance est apparue similaire pour la chlorophylle A & B, aussi bien que pour l'activité de l'acide  $\delta$ -aminolevulinique déhydratase (ALA-D). D'un autre côté la submersion a augmenté de façon significative la teneur en acide indolacétique (IAA) des parties aériennes des 2 plantes, mais la salinité a eu un effet décroissant. Les 2 traitements ont diminué les niveaux d'acide gibbérellique (GA<sub>3</sub>) dans les parties aériennes de *Vigna sinensis* ou *Zea mays*, ainsi que celui de zéatine dans celles de *Zea mays*. Pendant ce temps, l'acide abscisique (ABA) était accumulé en abondance dans les parties aériennes des plantes stressées. L'application foliaire de 50 ppm de kinétine s'est opposée à la réduction résultante de la croissance et de la teneur en chlorophylle des 2 plantes, mais a partiellement réduit l'inhibition de l'activité de ALA-D. De plus, la kinétine a augmenté IAA, GA<sub>3</sub> et la zéatine dans les plantes stressées jusqu'à atteindre pratiquement le niveau du témoin, mais a réduit de façon marquée ABA. Ces résultats indiquent que l'état des dommages et la restauration des conditions normales a été atteint soit partiellement, soit complètement en appliquant la kinétine. Ce rétablissement pourrait être une conséquence de plusieurs rôles joués par de telles hormones qui peuvent induire le déclenchement du métabolisme cellulaire interne et aussi induire des altérations dans les rapports des régulateurs de croissance.

régulateurs de croissance / salinité / *Vigna sinensis* / submersion / *Zea mays*

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## 1. INTRODUCTION

Plant growth regulators mainly control growth and development of germinating seedlings. As far as such substances are involved in the mechanism of seed germination, plant growth may depend on the ratio rather than on the absolute levels of growth substances in the plant. These ratios may be rapidly and dramatically changed in response to stress conditions imposed by waterlogging or salinity, thus leading to physiological disturbances associated with general reduction in growth. Nevertheless, Lerner and Amzallag [15] stated that plant response to salinity is not a function of the toxicity of ions but appears to be a consequence of the effect of salt on hormonal balance. In this connection, Wright [37] stated that the increase in ABA content of leaves from waterlogged plants appeared to be correlated with stomatal closure. However, Zhang and Zhang [41] found marked increases in ABA content of shoots and roots of waterlogged pea plants. Additionally, increases in the endogenous ABA levels were detected in roots of *Oryza sativa* L. exposed to 150 mM NaCl [21]. As yet, we know little of the physiological role of growth regulators, which have occasionally been reported to counteract the inhibitory effects induced by waterlogging and salinity on the growth and metabolism of treated plants. In this context, the suppression of growth parameters and pigment content as well as nitrogen, proline, Na<sup>+</sup> and ABA of peas in response to salinity treatments was nullified either partially or completely when seeds were presoaked in 100 ppm GA<sub>3</sub> [38]. However, waterlogging or salinity may inhibit net photosynthesis by low stomatal conductance, decrease in water and nutrient uptake, reduction in specific biochemical phenomena for carbon uptake, depression of photochemical capacity or some combination of these [1, 34, 40].

The objective of the present work was to study the possible effects of water stress imposed by waterlogging or salinization, using artificial seawater mixture, on growth and endogenous growth regulators of *Vigna sinensis* and *Zea mays* at the vegetative stage. We also focused on evaluating the role of kinetin application at 50 ppm as a foliar spray in overcoming the deterioratory effects that resulted from water stress treatments.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and apparatus

Authentic IAA, GA<sub>3</sub>, zeatin, ABA, ALA and porphobilinogen were obtained from Sigma (St. Louis, USA). All organic solvents were those specific for High Performance Liquid Chromatography (HPLC).

A Varian Model Cary 210 double-beam grating Spectrophotometer was used. The HPLC instruments were: (i) a Milton-Roy preparative HPLC assembled from a CM 4000 Pump, a Spectro Monitor 3100 Detector and a LC column, 25 cm × 20 mm i.d., Spherisorb ODS2, 10 μm Stagroma Wallisellen (CH), and (ii) a Perkin-Elmer analytical HPLC assembled from a Series 410 LC Pump, LC 235 Diode Array Detector interfaced with an Omega-2 Analytical Chromatographic

workstation (version 2.50 software) and an Omega 235 software upgrade kit (PE Nelson) equipped with a LC column, 25 cm × 4.6 mm i.d., C18 reversed phase with a Supelguard LC 18 guard column (Supelco Inc., Bellefonte, PA).

### 2.2. Plant materials and growth conditions

Pure strains of *Vigna sinensis* (southern pea, var. Cream 7) and *Zea mays* (var. Dentate, single cross 10) were obtained from the Agriculture Research Center, Ministry of Agriculture Giza, Egypt. The seeds, after surface sterilization, were thoroughly washed and soaked in distilled water. Thereafter, 12 and 10 seeds of *Vigna sinensis* and *Zea mays*, respectively, were sown separately in plastic pots (25 cm in diameter) containing equal amounts (2.5 kg) of a homogeneous mixture of sand and clay soil (1:2 w/w). Seedlings were then left to grow in a greenhouse under normal day/night conditions (at 24/16 °C day/night, with a 14-h photoperiod, 450–500 μmol photons·m<sup>-2</sup>·s<sup>-1</sup> photon flux density, and 75–80% relative humidity). Uniform watering was carried out according to the usual practice. Thinning was carried out after 14 days to leave 5 and 4 uniform plants of *Vigna sinensis* and *Zea mays*, respectively, per pot.

Depending upon a fully factorial design in triplicate, taking into consideration the subsequent sampling intervals, the pots for each species were then divided into 5 groups; one was kept to serve as a control and the others were used for waterlogging or irrigation by 1/4-strength seawater. Each treatment was performed either without or with the simultaneous foliar spray of 50 ppm kinetin; each pot took 100 ml. The arrangement of pots was changed and rotated daily to minimize the environmental variabilities. The water holding capacity of the soil, determined as g% of dry soil, was 30%. The water level in the waterlogged or salinized pots was, respectively, kept at 120% (saturated pots) or 60% of water field capacity. In a preliminary experiment, field capacity was measured and an amount of water equivalent to 60% or 120% of that previously remaining was added to soil to give 60% or 120% of field capacity. It was maintained through the experiment by adding the appropriate amount of water daily to every pot in order to keep its weight constant. In waterlogging treatments, demineralized water was used, containing 1/5-strength of the Pfeffer's nutrient mixture: [(mM) 4.89 Ca(NO<sub>3</sub>)<sub>2</sub>, 1.98 KNO<sub>3</sub>, 2.68 KCl, 1.48 KH<sub>2</sub>PO<sub>4</sub> and 1.67 MgSO<sub>4</sub>]. Microelements were supplied to the nutrient solution at concentrations as follows: [(μM) 46.1 H<sub>3</sub>BO<sub>3</sub>, 9.14 MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.32 CuSO<sub>4</sub>·5H<sub>2</sub>O, 1.37 ZnSO<sub>4</sub> and 0.5 H<sub>2</sub>MoO<sub>4</sub>·H<sub>2</sub>O]. In saline treatments, an artificial seawater mixture containing the following concentrations: [(mM) 460 Na, 360 Cl, 10 K, 10 Ca, 55 Mg, 28 S, 0.035 N, 0.002 P, 0.4 B, 0.024 Li, 0.07 F, 0.8 Br and 0.1 Si] were added to the nutrient solution. The pots were irrigated daily with equal and constant amounts either of fresh water (for control) or of sea water (for salt treatment). Demineralized water was added to compensate for water loss when necessary by maintaining a fixed constant weight of every pot containing wetted soil. Both species were subjected to both treatments for the subsequent 3 weeks. These intervals represented 14-, 17-, 21-, 24-, 28-, 31- and 35-day-old plants. The initial samples were taken at zero time from the thinned plants.

### 2.3. Determination of pigments

Chlorophyll a and b were determined in the fresh tissues after extraction with 85% acetone according to the spectrophotometric method described by Metzner et al. [19].

### 2.4. Extraction and assay of ALA-D

Samples were homogenized with chilled acetone, filtered through a Büchner funnel and washed with chilled acetone. The residues were spread on filter papers and allowed to dry at room temperature. An aliquot of the acetone powder (0.5 g) was mixed with 25 ml of 0.05 M Tris-HCl buffer (pH 9.0) for 10 min at 4 °C and then centrifuged at 48 200 g for 15 min at 4 °C; the supernatant was used as enzyme extract. ALA-D activity was assayed in 4 ml of incubation mixture containing 3.6 ml of enzyme extract and 0.4 ml of 5 mg/ml ALA in 0.05 M Tris-HCl buffer (pH 7.0). The mixtures were incubated at 34 °C for 1 h, then stopped by adding 2 ml of 0.1 M HgCl<sub>2</sub> in 10% CCl<sub>3</sub>COOH. The quantity of PBG formed was determined spectrophotometrically by reaction with dimethylaminobenzaldehyde at 555 nm [18].

### 2.5. Determination of ABA and IAA

ABA and IAA were extracted, purified, and estimated according to the method of Majcherczyk et al. [17]. Plant tissues were homogenized with acetonitrile containing 2,6-ditertiary butyl-p-cresol (3 × 25 ml) to extract ABA and IAA. Partitioning with chloroform was used for hormone clean-up. Fractions corresponding to IAA and ABA were separated using the preparative HPLC and then submitted to complete dryness. Methanol (500 µl) was added as diluent and only 6 µl were injected onto the analytical HPLC. The conditions of the analysis were: the mobile phase was methanol containing 0.05% acetic acid/water containing 0.05% acetic acid (20/80, v/v), linear gradient over 30 min to the final concentration 70/30 (v/v), the flow rate was 1 ml·min<sup>-1</sup>, wavelength was 255 nm, and the retention time for ABA and IAA was 27.4 min and 22.5 min, respectively.

### 2.6. Determination of GA<sub>3</sub>

GA<sub>3</sub> was extracted, purified and estimated according to the procedure of Lin and Stafford [16]. Plant tissues were homogenized with methanol (3 × 25 ml) to extract GA<sub>3</sub>. Purification was carried out by partitioning with hexane, n-butanol, ethyl acetate, and finally with polyvinyl polypyrrolidone. GA<sub>3</sub> was separated with the preparative HPLC and then submitted to complete dryness. Methanol (100 µl) was added as diluent and only 6 µl were injected onto the analytical HPLC. The conditions of the analysis were: the mobile phase was methanol containing 0.05% acetic acid/water (20/80, v/v), linear gradient over 20 min to the final concentration 60/40 (v/v), the flow rate was 1 ml·min<sup>-1</sup>, wavelength was 255 nm, and the retention time was 17 min.

### 2.7. Determination of Zeatin

Zeatin was extracted, purified and estimated following the method of Schwartzberg et al. [31]. Plant tissues were

homogenized with boiling ethanol (3 × 25 ml) to extract zeatin. Purification was carried out by partitioning against petroleum ether, DEAE-Cellulose Column and Sep-pak Cartridge. Zeatin was separated by the preparative HPLC, and then submitted to complete dryness. Methanol (100 µl) was added as diluent and only 6 µl were injected onto the analytical HPLC. The conditions of the analysis were: the mobile phase was methanol/ammonium formate, pH 3.7 (20/80, v/v), isocratic elution over 8 min, the flow rate was 1 ml·min<sup>-1</sup>, wavelength was 255 nm, and the retention time was 6.0 min.

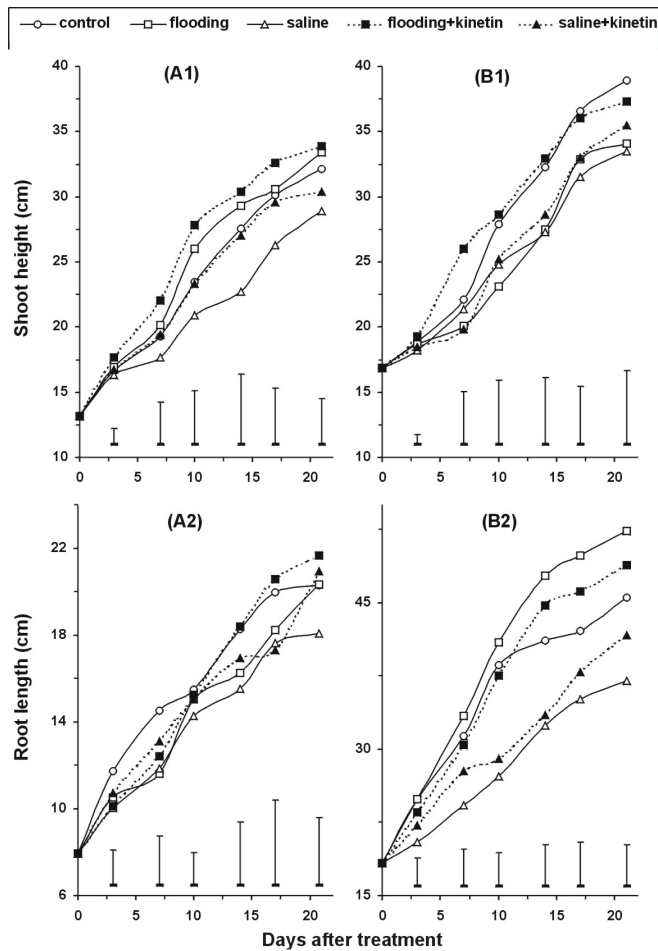
### 2.8. Growth parameter measurements

In treated and untreated samples, shoot height (as soil surface to tip) and root length (as depth of the root front) were measured in cm. Dry weight of shoots and roots was conducted on a plant number basis by drying a known amount of each tissue at 80 °C for 48 h and then keeping it in a desiccator until it reached a constant weight.

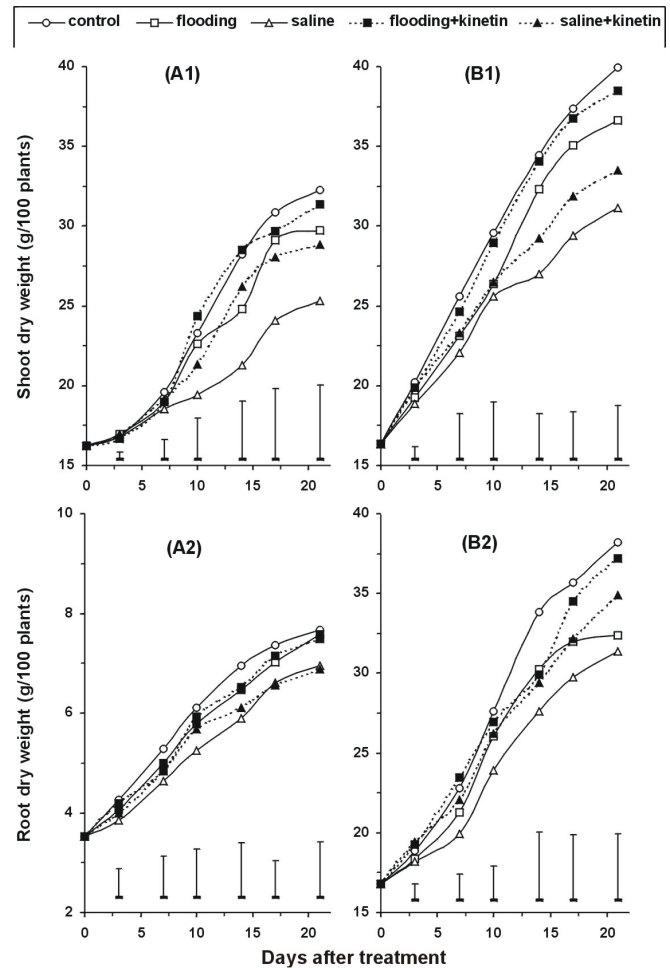
Chemical analyses of chlorophyll content and ALA-D activity were carried out on plant leaves throughout the 6 different intervals of growth, whereas growth parameters were measured on shoots and roots while plant growth regulators were determined on shoots. Triplicate determinations were conducted throughout the 6 different intervals of growth and only the mean values are presented. The full data were statistically analyzed, in order to compare between the mean values at the same sampling interval with their respective control, using the least significant difference (L.S.D) test at the 5% level [32].

## 3. RESULTS

As is apparent from Figure 1, the shoot height of *Vigna sinensis* and *Zea mays* plants was slightly affected by treatment with either waterlogging or seawater irrigation; insignificant increases or decreases were, respectively, detected throughout the various growth intervals. Meanwhile, root length of treated *Vigna sinensis* plants was significantly reduced during the first week; thereafter, the effect of waterlogging appeared non-significant. On the other hand, root length of waterlogged *Zea mays* plants was non-significantly affected up to the 10th day after treatment, then a significant increase was recognized throughout the subsequent intervals. On the contrary, root length of samples subjected to seawater was significantly decreased to below control values throughout the experimental period. Foliar application of 50 ppm kinetin to both species under these conditions appeared to variably change plant height compared with non-kinetin-treated species. Flooding effects seemed to have been accelerated in the presence of kinetin. In addition, shoot height of salinized plants became very close to control values during the whole experiment, indicating complete nullification of the reduction effect induced by salinization. Similarly, kinetin seemed to counterbalance the reduction effect maintained in root length of *Vigna sinensis* under waterlogging or salinity. Hence, root length of these treated plants under kinetin application was more or less comparable with untreated plants. On the other hand, kinetin appeared to induce slight, if any changes in root length of



**Figure 1.** The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on shoot height (1) and root length (2) of *Vigna sinensis* (A) and *Zea mays* (B) plants grown in pots. The data presented are the means of triplicate determinations. Vertical bars represent LSD at 5% level.

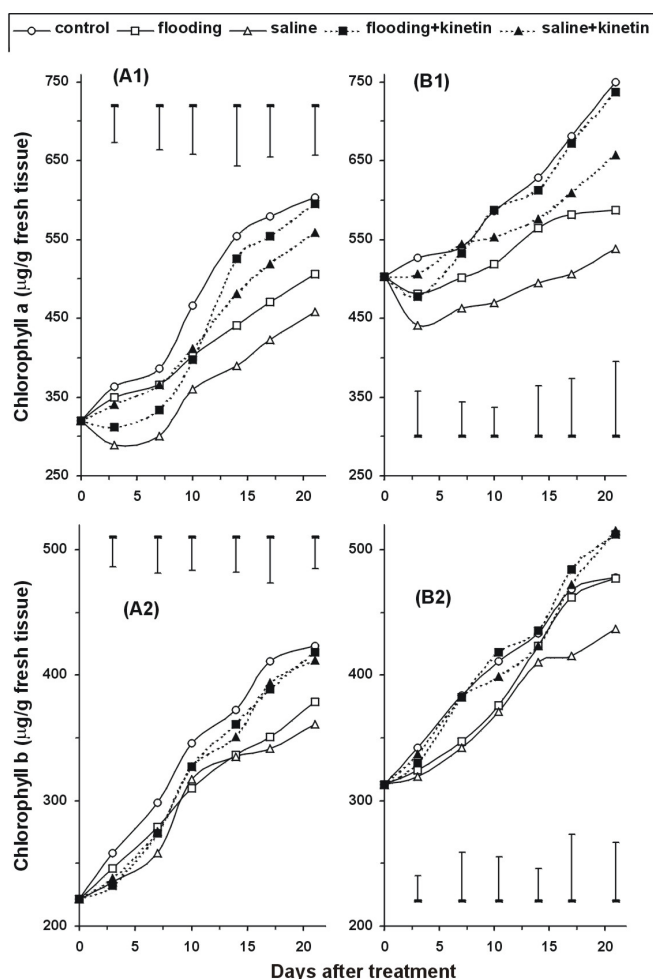


**Figure 2.** The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on dry matter content of shoots (1) and roots (2) of *Vigna sinensis* (A) and *Zea mays* (B) plants grown in pots. The data presented are the means of triplicate determinations. Vertical bars represent LSD at 5% level.

waterlogged *Zea mays*, but partially nullified the salinity-induced inhibitions.

As shown in Figure 2, dry weights of *Vigna sinensis* or *Zea mays* shoots appeared to be non-significantly affected by waterlogging. Salinity, however, even though it did not affect root dry weight of *Vigna sinensis*, significantly reduced shoot dry matter to below control levels from 10 days onwards. In *Zea mays*, significant reductions were found in dry weight of shoots and roots in response to salinization throughout the experimental period. Spraying stressed plants with kinetin appeared to counteract the observed decreases in dry matter throughout the growth intervals. Thus, dry matter values of shoots became comparable with control values under the combination of kinetin with either waterlogging or seawater irrigation during the whole experiment. A partial nullification of the reduction effects of stress conditions on dry matter accumulation appears to have been operative throughout the experimental period.

Figure 3 shows that the pattern of changes in chlorophyll a in leaves of treated plants appeared in general alike to that of chlorophyll b. Whereas salinity significantly decreased the contents of chlorophyll a of *Vigna sinensis* or *Zea mays* during the entire experiment, waterlogging had a non-significant effect during the first week of treatment; thereafter, significant decreases set in. In addition, waterlogging did not significantly change chlorophyll b content of leaves during the first 2 weeks of treatment, then a significant decrease was detected afterwards. Salinity, however, significantly reduced chlorophyll b content throughout the experimental period. In *Zea mays* leaves, chlorophyll b was not changed by waterlogging but significantly reduced by salinity during the first 10 days after treatment. The exogenous application of kinetin to the waterlogged or salinized plants induced variable partial nullification of the inhibitory effects of the stress treatments on chlorophyll a content. Similarly, chlorophyll b in the stressed plants appeared more or less comparable with the respective control

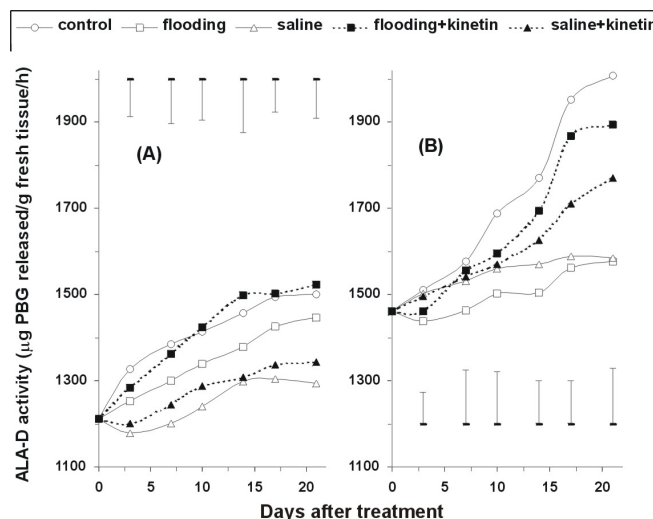


**Figure 3.** The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on contents of chlorophyll a (1) and chlorophyll b (2) in leaves of *Vigna sinensis* (A) and *Zea mays* (B) plants grown in pots. The data presented are the means of triplicate determinations. Vertical bars represent LSD at 5% level.

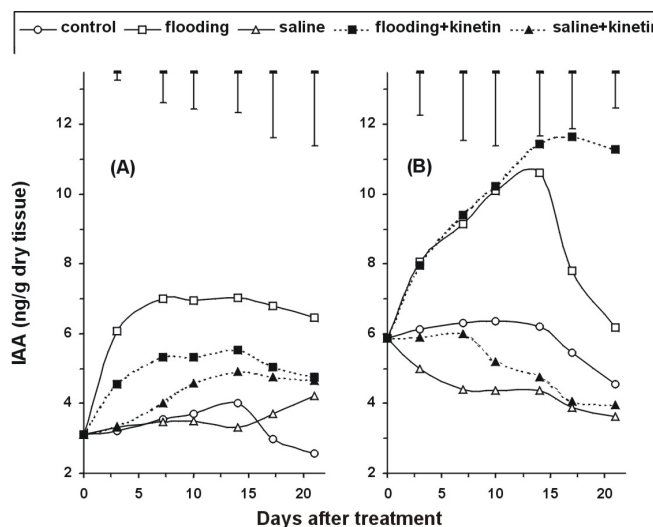
after kinetin application, indicating great nullification of the reduction effect of waterlogging or salinity.

From the data illustrated in Figure 4, the activity of ALA-D in shoots of *Vigna sinensis* was not significantly affected by waterlogging but significantly decreased by salinity. On the other hand, both treatments exerted their significant reductions on ALA-D activity in shoots of treated *Zea mays* after one week of treatment. Foliar application of kinetin to *Vigna sinensis* or *Zea mays* plants appeared to completely nullify the observed decreases in ALA-D activity in waterlogged plants. On the other hand, although the ALA-D activity levels of both species in response to kinetin application in combination with salinity appeared to increase, the activity levels remained significantly lower than control levels during the whole experiment.

It is apparent from Figure 5 that waterlogging induced a significant accumulation of IAA in shoots of *Vigna sinensis* and *Zea mays* plants as compared with the respective normal

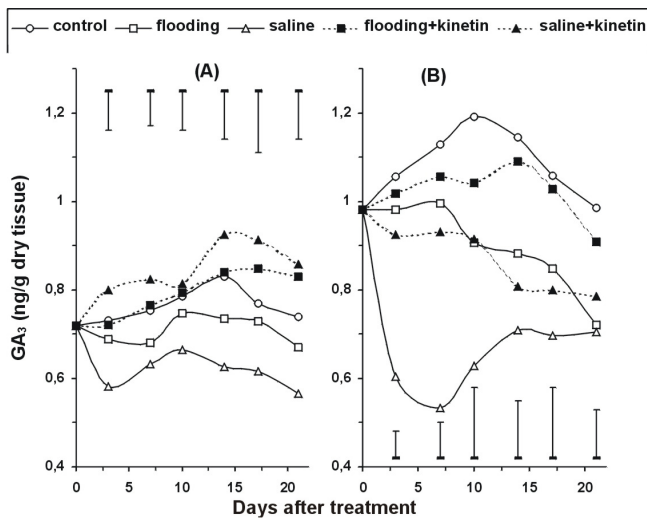


**Figure 4.** The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on ALA-D activity in leaves of *Vigna sinensis* (A) and *Zea mays* (B) plants grown in pots. The data presented are the means of triplicate determinations. Vertical bars represent LSD at 5% level.



**Figure 5.** The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on IAA contents in shoots of *Vigna sinensis* (A) and *Zea mays* (B) plants grown in pots. The data presented are the means of triplicate determinations. Vertical bars represent LSD at 5% level.

growing plants. The accumulation effect was abrupt and sharp in *Vigna sinensis* during the first week, followed by leveling off during the rest of the experimental period. While in *Zea mays*, IAA showed a sharp progressive increase up to the 14th day after treatment followed afterwards by a progressive sharp decline. On the other hand, salinity insignificantly changed the IAA content of both species throughout the experimental period. Foliar spraying of kinetin to treated *Vigna sinensis* caused a decline in IAA levels of waterlogged plants but



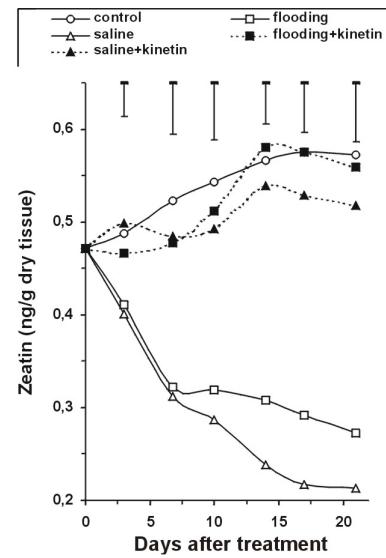
**Figure 6.** The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on GA<sub>3</sub> contents in shoots of *Vigna sinensis* (A) and *Zea mays* (B) plants grown in pots. The data presented are the means of triplicate determinations. Vertical bars represent LSD at 5% level.

these levels remained, in general, significantly higher than those of controls. Although the levels of saline-treated *Vigna sinensis* plants were slightly increased as a result of exogenous application of kinetin, these levels did not differ significantly from controls throughout the experimental period. On the other hand, kinetin application to *Zea mays* plants did not change the effect of waterlogging on IAA levels but seemed to partially nullify the retarding action of salinity.

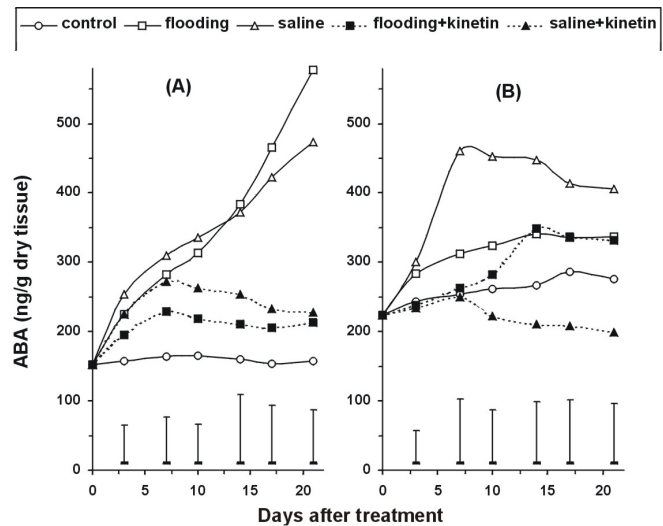
The results in Figure 6 show that waterlogging *Vigna sinensis* or *Zea mays* plants induced, respectively, non-significant or significant decreases in GA<sub>3</sub> levels in relation to control levels. On the other hand, salinization significantly decreased GA<sub>3</sub> content in shoots of both species throughout the experimental intervals. Exogenous application of kinetin to *Vigna sinensis* under waterlogging or salinity not only nullified the inhibiting actions of these treatments but also induced variable increases in GA<sub>3</sub> contents above the values of controls. Whereas spraying kinetin on treated *Zea mays* plants appeared to partially counterbalance the observed decreases in GA<sub>3</sub> contents in waterlogged and saline-treated plants.

In this investigation zeatin was recorded only in shoots of *Zea mays* plants but not in *Vigna sinensis*, which contained negligible amounts that could hardly be detected in the present situation. It is apparent from Figure 7 that waterlogging as well as salinity provoked a significant decline in zeatin levels in *Zea mays* shoots. This decline seemed to be continuous up to the end of the experimental period. As can be seen from the figure, kinetin application to the stressed plants greatly nullified the inhibitory action of waterlogging and of salinization on zeatin content throughout the experimental period.

It is obvious from Figure 8 that waterlogging or salinity throughout the experimental intervals progressively increased ABA content of *Vigna sinensis* plants. Whereas in waterlogged *Zea mays*, ABA content was consistently higher than



**Figure 7.** The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on zeatin contents in shoots of *Zea mays* plants grown in pots. The data presented are the means of triplicate determinations. Vertical bars represent LSD at 5% level.



**Figure 8.** The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on ABA contents in shoots of *Vigna sinensis* (A) and *Zea mays* (B) plants grown in pots. The data presented are the means of triplicate determinations. Vertical bars represent LSD at 5% level.

that of controls. In saline-treated plants, a sharp increase in ABA content was apparent during the first 7 to 14 days of the experimental period followed by a decline afterwards; these contents, however, appeared to be significantly higher than the level of control. Foliar application of kinetin caused a sharp decline in ABA content of treated *Vigna sinensis*. On the other hand, kinetin induced partial nullification of ABA content in shoots of waterlogged *Zea mays*; these contents became slightly higher than those of control. Whereas in salinized

plants, not only complete nullification of the increasing effect of these treatments on ABA was induced but also an appreciable decrease to a value below those of controls was maintained throughout the experimental period.

#### 4. DISCUSSION

In the present investigation, the stress treatments induced remarkable variations in growth criteria of both species. The first sign was detected as disturbances in plant morphology. Waterlogging, in general, non-significantly affected the root length of both species whereas salinity led to a dominant decrease in shoot height and root length. In this regard, Whitlow and Harris [36] reported that flooding modifies almost every aspect of shoot behavior. It is also stated that flooding injury is initiated when the concentration of molecular oxygen in the rooting zone is too low to maintain aerobic respiration throughout the root tissues. Below this critical oxygen pressure every metabolic aspect in the cell progressively declines with inhibition of root growth, ion transport and alterations in water and hormone relations. Waterlogging generally inhibited net photosynthesis by several mechanisms [1]. Plant length of several species was also shown to be decreased in response to salinity [8, 40]. The present results also showed great reductive effects for salinity treatments on dry weights in shoots and roots of both species. In accord with these results, Younis et al. [39], using flax, cotton and castor bean, Pezeshki et al. [26], using *Avicenna germinans*, and Sanchez-Blanco et al. [30], using *Lotus creticus*, recorded that salinization resulted in a reduction in dry matter accumulation of tested species.

The decline in dry matter accumulation in the present results might be attributed to reduction in water uptake and to inhibition of photosynthetic output and carbohydrate syntheses. In this respect, Sultana et al. [34] found that salinity reduced photosynthesis and dry matter accumulation in the salinized *Oryza sativa*. The reduction in photosynthesis was attributed to a reduction of available CO<sub>2</sub> by stomatal closure, cumulative effects of leaf water and osmotic potential, stomatal conductance, transpiration rate, relative leaf water content and biochemical constituents such as photosynthetic pigments, carbohydrates and protein [7, 26, 34, 40].

Again, the observed reduction in growth criteria of *Vigna sinensis* and *Zea mays* in response to treatment with salinity might be attributed to imbalance in internal contents of plant growth regulators. Thus, a close relationship appeared to exist between the changes in growth criteria and the endogenous levels of growth hormones (Tab. I). The predominant reduction in the growth parameters in response to salinity can be attributed to the increase in ABA level and the decreases in levels of auxins, gibberellins and cytokinins. The results indicated that the growth parameters of the stressed plants showed variable responses (varying between slight to partial or complete nullification) to the foliar application of kinetin. Thus, the inhibitory effects caused by waterlogging on the dry weights of shoots and roots of both species appeared to be nullified by kinetin either completely (in *Zea mays*) or partially (in *Vigna sinensis*).

**Table I.** Correlation coefficient between dry matter content and levels of endogenous plant growth regulators as well as between chlorophyll a content and ALA-D activity in shoots of *Vigna sinensis* and *Zea mays*.

	Dry matter content against				Chlorophyll a content against
	IAA	GA <sub>3</sub>	zeatin	ABA	ALA-D
<i>Vigna Sinensis</i>					
Waterlogging	57	56	/	93	62
Salinity	32	71	/	78	73
<i>Zea mays</i>					
Waterlogging	66	82	91	59	94
Salinity	61	58	96	57	53

In agreement with our results, Das Gupta et al. [5] recorded that foliar application of plant growth regulators such as IAA, GA<sub>3</sub> and kinetin helped to re-establish water content in mung bean (*Vigna radiata*) plants which had been retarded by severe water deficits. The role of growth substances in overcoming the effects of salinity on growth may be due to the changes in endogenous cytokinins, which affect plant water balance and/or decreasing root resistance to water flow. Also, Muir and Cheng [22] showed that both zeatin and GA<sub>3</sub> caused an increase in fresh weight and area of the cotyledons of cucumber.

Detecting the changes in chlorophyll content, which might point to leaf senescence, could also indicate visual symptoms of stress. Chlorophyll a and b in leaves of *Vigna sinensis* and *Zea mays* were non-significantly changed by waterlogging but significantly decreased by salinity. These changes lead us to suggest that both species have some affinity to tolerating waterlogging but not saline stress. In this connection, it was reported that waterlogging or salinity decreased the content of photosynthetic pigments in several plant species [1, 6, 34]. Moreover, Strogonov [33] proposed that the reduction in pigment contents in response to salinization is probably due to the inhibitory effect of the accumulated ions of the various salts on the biosynthesis of the different pigment fractions. On the other hand, Yu and Rengel [40] concluded that salt stress exposes chloroplasts to excessive excitation energy, thus leading to oxidative stress. They furthermore indicated that Cl<sup>-</sup> toxicity disrupts normal electron flow to PSII. Such disruption might result in excessive electron leakage, which in turn could increase the generation of reactive oxygen species (inevitable by-products of cell metabolism attacking lipid, protein and nucleic acids).

Examination of the data revealed a close correlation between the changes in ALA-D activity and chlorophyll content (Tab. I). Thus the inhibition in the enzyme activity appeared to coincide with the decrease in the chlorophyll content in stressed plants. The physiological and biochemical function of ALA-D is believed to be the synthesis of the porphyrin ring, which is utilized in the synthesis of chlorophyll, peroxidase, catalase and cytochrome. Thus, the conditions which block ALA-D synthesis will subsequently interfere with chlorophyll synthesis. The inhibitory effects of water



stress as induced by salinity or waterlogging on chlorophyll a and b were partially nullified when the treated plants were sprayed with kinetin. In accord with these results, it has been found that the inhibitory effects of water stress on photosynthetic pigments in green shoots of some species were generally counteracted by the exogenously-applied phytohormones [25, 38]. Of further interest in this connection, Osborne [24] reported that exogenous cytokinins promoted retention of chlorophyll and delayed leaf senescence induced by flooding. Moreover, Sakr [29] also showed that kinetin or GA<sub>3</sub> partially overcome the inhibitory effects of CaCl<sub>2</sub> and NaCl in decreasing photosynthetic pigments in the cotyledonary leaves of cotton seedlings. Because the flooding reduces the flow of cytokinins to shoots, it is reasonable to hypothesize that flooding-induced chlorosis may in part be due to cytokinin starvation. Supplementation of cytokinin could eventually increase the number of chloroplasts in the leaf by increasing both intensity of cell growth phytohormones and the activity of cytoplasm ribosomes, and consequently stimulate chlorophyll synthesis [3].

It has been suggested that when roots are generally stressed, the amounts of hormone or hormone precursors entering the shoots can be altered. These changes may constitute a physiologically active message that would modify shoot physiology and development. The present results revealed that waterlogging increased IAA contents of *Vigna sinensis* and *Zea mays*. Similarly, Phillips [27] found a three-fold increase in auxin content of sunflower after 14 days of flooding. Thus, flooding could affect auxin levels by interfering with auxin production in roots, or with its transport. Indeed, Wample and Reid [35] recorded that auxin transport in sunflower from shoots to roots stopped in response to waterlogging and the hormone was accumulated in the shoot system. They further indicated that flooding inhibited the basipetal movement of <sup>14</sup>C-IAA and slowed its breakdown. They concluded that inhibition of both transport and metabolism might have caused the flooding-induced auxin accumulation. In addition, elevated ethylene levels in flooded plants could inhibit auxin transport, and thus slow the movement of auxin from shoots to roots [11].

Meanwhile, there was a marked reduction in GA<sub>3</sub> in both species. In this connection, reduced levels of cytokinins have been detected in the xylem sap of flooded sunflower plants [4]. As the biosynthesis and transformation of gibberellins and cytokinins take place in roots, flooding can thus directly modify their production. Reid and Crozier [28] suggested that the lowered level of GA<sub>3</sub> and cytokinins in shoots of flooded tomato plants might result from the reduced export from roots to shoots. In addition, Kord and Khalil [14] concluded that salinity reduces hormone delivery from roots to leaves. Moreover, Lerner and Amzallag [15] stated that salt toxicity is a consequence of the effect of salt on hormonal balance. In the present investigation, seawater did not significantly affect the IAA level in *Vigna sinensis* and *Zea mays* shoots but significantly decreased GA<sub>3</sub> in both species as well as zeatin in *Zea mays*. Supporting these findings, Ghazi [10] found a reduction in IAA, GA<sub>3</sub> and cytokinin contents of saline-treated *Vicia faba* plants. In addition, Mohamed [20] recorded that irrigation of wheat plants with NaCl decreased the total auxins, gibberellins and cytokinin contents in developing wheat grains. Various explanations for the observed reduction in the cytokinins

in salinized plants have been reported. It could be suggested that salinization may change cytokinins from the free active to bound inactive form [9]. Alternatively, Itai and Vaadia [13] postulated that cytokinin biosynthesis in roots may cease at the moment the water tension in the leaf is enhanced.

The observed accumulation of IAA in response to waterlogging was accompanied by marked induction of ABA. The elevated ABA level could be the result of an effect of flooding on the direction and rate of transport, rates of synthesis and destruction and even on binding and release. Thus, ABA is synthesized in the roots of several species in response to different stresses [2, 23]. It has been suggested that acclimatization of ABA is a natural response that mediates the degree to which the plant can respond to environmental stress [21]. Moreover, Zhang and Zhang [41] found that ABA content in shoots and roots of flooded pea plants (*Pisum sativum* L.) increased up to eight-fold. They observed that ABA increase in old leaves preceded that in young leaves, suggesting that the flooding-induced ABA mainly results from wilting of old leaves. In this context, flooding was found to cause rapid wilting in a wide range of species [12].

In this work, an attempt has been made to employ kinetin as an active phytohormone for overcoming the growth modifications of both species that result from waterlogging or salinity. The results revealed that kinetin mostly nullified the varied changes in the different growth regulators of the stressed *Vigna sinensis* and *Zea mays* plants. Therefore, it became evident that the exogenous application of kinetin appeared to supply more or less sufficient quantities which were involved in the recovery of growth under stress as induced by waterlogging and salinization. This recovery may be a consequence of several roles played by such hormones, which can cause triggering of the internal cellular metabolism and also induce alterations in the ratios of the growth regulators which have been shown to be critical determinators of growth and differentiation.

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