

Effect of Kinetin on Photosynthetic Activity and Carbohydrate Content in Waterlogged or Seawater Treated *Vigna sinensis* and *Zea mays*

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Abstract: Waterlogging decreased carotenoids content of *Vigna sinensis* and *Zea mays* whilst salinity provoked increases in former and decreases in later. Both treatments showed significant reductions in chlorophylls content and in photosynthetic activity (Hill reaction and $^{14}\text{CO}_2$ -light fixation). A significant decrease in the activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was detected in both plants. These reductions could result in losses in photosynthetic activity with a drop in the net formation of carbohydrates. Waterlogging markedly increased glucose and sucrose in shoots and roots of both plants throughout the experimental period but greatly decreased polysaccharides, whilst salinity reduced all carbohydrate fractions. Foliar application of 50 ppm kinetin to treated plants mostly counterbalanced the observed changes in pigments as well as in Hill reaction and $^{14}\text{CO}_2$ assimilation. Also kinetin rendered levels of carbohydrate fractions in shoots and roots of treated plants and activity of Rubisco most likely to reach control values. The increased levels of glucose and sucrose concomitant with decreased polysaccharides might point to a block in their transport rather than from an over production. Kinetin counterbalanced the effects of waterlogging or salinity on photosynthetic activity, probably through more production of enzyme and/or delay of senescence.

Key words: Carbohydrates, photosynthetic activity, salinity, *Vigna sinensis*, waterlogging, *Zea mays*

Introduction

Growth and distribution of plants are affected chiefly by too little or too much water. Expansive growth is an overriding physiological event, which can be considered as the combined result of the influence of the environmental and metabolic events on the overall plant productivity. Water stress, induced either by waterlogging or salinization affects practically every aspect of plant growth. Photosynthesis is an important process, which can be altered by waterlogging or salinity stress. A decrease in CO_2 assimilation was induced by waterlogging (Younis *et al.*, 1993 and Habib-ur-Rehman, 1999) and salinity (Sultana *et al.*, 1999). Photosynthetic pigments and certain enzymes are important in driving photosynthesis. Of these enzymes ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) being the most essential one. Rubisco is by far the most abundant protein present in green leaves of higher plants (Spreitzer, 1993) catalyzes two reactions, the carboxylation and oxygenation of ribulose-1,5-bisphosphate. The principal endproducts of photosynthetic carbohydrate metabolism are starch and sucrose. Stress can lead to change in carbohydrates production. Carbon metabolism may have some contribution to salinity tolerance in plant (Rout and Shaw, 1998). Decrease in glucose, fructose, polysaccharides and total carbohydrates were induced in *Vicia faba* shoots by waterlogging (El-Shahaby *et al.*, 1994) and *Phaseolus vulgaris* in shoots and roots by salinity (Younis *et al.*, 1993). On the other hand, Imamul Huq and Larher (1983) found that salinity increased total soluble sugars in *Phaseolus aureus* shoots. Similar increase in total carbohydrates of alfalfa roots were also induced by salinity (Fougere *et al.*, 1991).

Therefore the objective of this work was to study the possible effects of waterlogging or salinization using artificial seawater on photosynthetic activity of *Vigna sinensis* and *Zea mays* at the vegetative stage. Also, a particular interest was focussed to evaluate the role of kinetin application at 50 ppm as a foliar spray in overcoming the deleterious effects of stress treatments.

Materials and Methods

Plant materials and growth conditions: Pure strains of *Vigna sinensis* (southern pea, var. Cream 7) and *Zea mays* (single cross 10, var. Dentate) were obtained from the Agriculture

Research Center, Ministry of Agriculture, Egypt. The seeds, after surface sterilization, were thoroughly washed and then 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). The seedlings were then left to grow in a green house under normal day/night conditions. Uniform watering was carried out. Thinning was carried out after 14 days to leave 5 and 4 uniform plants of *Vigna sinensis* and *Zea mays* respectively per pot.

The pots used for each plant were then divided into 5 groups; one was kept to serve as a control and the others were used for waterlogging or irrigation by 3-strength of seawater. Each treatment was performed either with or without simultaneous foliar spray of 50 ppm kinetin; each pot took 100 ml. Water level in the logged or salinized pots was respectively kept at 120% or 60% of water field capacity of soil. Water holding capacity of the used mixture of soil, determined as g% of dry soil, was 30%. In the logging treatments, water was used containing 1/5-strength of the Pfeffer's nutrient mixture: [(g/l) 0.8, $\text{Ca}(\text{NO}_3)_2$; 0.2, KNO_3 ; 0.2, KCl, KH_2PO_4 ; and 0.2, MgSO_4]. Microelements were supplied to the nutrient solution at concentrations as follows: (mg/l) 2.86, H_3BO_3 ; 1.81, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 0.08, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 0.22, ZnSO_4 and 0.09, $\text{H}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$. In the saline treatment, 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 was added to the nutrient solution. Water was added to compensate for transpirational water loss when necessary. The two investigated plants were subjected to the treatments for the subsequent 3 weeks and samples were collected after 3, 7, 10, 14, 17 and 21 days from treatment. These intervals represented 17-, 21-, 24-, 28-, 31- and 35- day-old plants. The initial samples were taken at zero time from the thinned plants.

Chemical analyses of pigments, Hill reaction, soluble and insoluble metabolites of CO_2 -light fixation and carbohydrate fractions as well as activity of Rubisco were performed. Samples were taken in triplicates and only the mean values are presented. The full data were statistically analyzed using the least significant difference (L.S.D) test at 5% level.

Determination of pigments: Contents of chlorophylls and carotenoids were determined in the fresh tissues after extraction with 85% acetone according to the spectrophotometric method described by Metzner *et al.* (1965).

Determination of photosynthetic activity: Hill reaction was assayed according to the procedures described by Arnon (1949). Photosystem II activity, as indicated by the rate of 2,6-dichlorophenolindophenol (2,6-DCPIP) photoreduction (Trebst, 1972) was monitored at 600 nm using spectrophotometer.

Photosynthetic activity ($^{14}\text{CO}_2$ -light fixation) was determined by introducing a definite fresh weight of leaves into the fixation apparatus as described by Shaddad (1979).

Estimation of soluble sugars: Sugars were extracted by overnight submersion of dry powders in 80% (v/v) ethanol at 25°C with periodic shaking. Glucose estimations were performed in the hood using a modification of O-toluidine procedure (Feteris, 1965). Absorbance was measured at 630 nm. Sucrose contents were determined by first degrading reactive sugars present in 0.1 ml extracts with 0.1 ml 5.4 N KOH at 97°C for 10 min. (Handel, 1968). Polysaccharides were determined in the dried plant residue, remaining after alcoholic extraction of soluble sugars, by heating under reflux in 1.5 N H_2SO_4 for 4 h at 100°C.

Assay of Rubisco: The procedures of Keys and Parry (1990) were followed for extraction and assay methods.

Results and Discussion

Salinity significantly decreased the contents of chlorophyll a+b of *Vigna sinensis* and *Zea mays* during the entire experiment (Fig. 1). On the other hand, waterlogging had no significant effects on *Vigna sinensis* during the first 10 days of treatment, thereafter significant decreases were set in. For *Zea mays*, these contents being unchanged during the whole experiment. Exogenous application of kinetin to the logged or salinized plants induced partial nullification of the inhibitory effects on chlorophylls content. In general, waterlogging or salinity non-significantly changed carotenoids content of *Vigna sinensis* but had a significant decreasing effect in *Zea mays* throughout the whole experiment. Partial nullification of the changed carotenoid values maintained in the stressed plants was induced by kinetin. Similar reductions in chlorophyll contents were observed in *Argyranthemum coronopifolium* plants in response to salt stress (De Herralde *et al.*, 1998).

The exogenously applied phytohormones generally counteracted the inhibitory effects of water stress on accumulation of photosynthetic pigments in shoots of a variety of species (Parthier, 1986).

Salinity induced significant reductions (Fig. 2) in the rate of Hill reaction, (Photosystem II) as indicated by the rate of photoreduction of 2,6-dichlorophenol indophenol (2,6-DCPIP), in *Vigna sinensis* leaves throughout the whole experiment while waterlogging only had a significant effect at 10th and 14th days of the experimental time. In *Zea mays*, both treatments induced a significant depression in Hill reaction activity during the entire experimental period. Kinetin sprays to treated plants appeared to counteract the deleterious effect of stress treatments on Hill reaction activity. In addition, Table 1 shows that photo assimilation of $^{14}\text{CO}_2$ into soluble photosynthetic metabolites in *Vigna sinensis* were significantly decreased by waterlogging. Salinization induced a lesser significant, if any, decreases in soluble photosynthetic metabolites of *Vigna sinensis*. These metabolites were significantly decreased in salinity-treated but slightly affected in waterlogged *Zea mays*. It is also indicated that, the patterns of changes in the insoluble photosynthetic intermediates

appeared more or less comparable with those of soluble ones with some tendency of *Zea mays* to tolerate salinity. As a whole total summations of photosynthetic intermediates in leaves of both treated plants appeared to be comparable with soluble fractions. Foliar application of kinetin seemed to completely overcome the deleterious effects of logging or salinity on levels of soluble and insoluble, and certainly total, photosynthetic metabolites in treated plants.

These results are in agreement with those obtained by Allakhverdiev *et al.* (2000) who indicated that salinity in activated PSI and PSII in *Syechococcus*. They further found that photosynthetic activity and cytoplasmic volume returned to the original values after removal of osmotic stress. In addition, Sultana *et al.*, (1999) reported that salinized *Oryza sativa* plants showed a decreased photosynthetic rate and consequently dry matter accumulation. In accordance, Younis *et al.* (1993) indicated that water regime or excessive water supply as well as different levels of salinity significantly reduced the photoreduction of DCPIP (Photosystem II activity) and total photosynthates of *Vicia faba*.

Furthermore, Habib-ur-Rehman (1999) reported that waterlogging significantly reduced net photosynthesis of maize plants. These findings may suggest that excessive supply of water could inhibit photosynthesis probably by inducing stomatal closure or by affecting the photosynthetic machinery at the mesophyll and chloroplast levels. Moreover, the results herein reported emphasized that under flooding or salinity, the rate of transformation of soluble photosynthates into insoluble ones was inhibited in both plants. Exogenous application of kinetin to the stressed plants greatly nullified the observed decline in photosynthetic activity. In this respect, Bradford (1982) found that supplementary cytokinins maintained the assimilative capacity of flooded plants. Also, Aldesuquy and Gaber (1992) stated that pretreatment of seeds with kinetin induced an increase in photosynthetic capacity by increasing Hill reaction activity of chloroplasts and soluble photosynthates in bean plants irrigated with seawater. It became evident that exogenous application of kinetin appeared to supply more or less sufficient quantities which were implicated in the recovery of growth under conditions of water stress. This recovery may be a consequence of several roles played by such a hormone 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.

Waterlogging resulted in a significant increase in glucose content in shoots of both plants from the 10th day in *Vigna sinensis* and throughout the whole experiment in *Zea mays* (Fig. 3). On the other hand, salinity significantly decreased glucose content of *Vigna sinensis* shoots during the experimental period but it had no effect in *Zea mays* shoots. Foliar application of kinetin to treated *Vigna sinensis* plants unchanged the effect of waterlogging and completely counteracted the inhibitory action of salinity. Similarly kinetin mostly lowered the effect of logging on glucose content in shoots of maize plants. In roots of both plants, waterlogging significantly stimulated glucose accumulation during the entire experiment while salinity showed low levels. Application of kinetin to treated plants decreased the accumulation of glucose in roots of waterlogged plants whereas the induced reduction in roots of salinized plants, if any, was completely nullified.

Waterlogging did not change sucrose content in shoots of both plants while salinity induced significant reductions (Fig. 4). Application of kinetin significantly increased sucrose content in shoots of logged *Vigna sinensis* but rendered the reductions of salinized plants to become non-significant only from the 14th day. Kinetin did not change sucrose levels in waterlogged *Zea mays*, so that levels in treated shoots remained

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Table 1: The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on photosynthetic assimilation of $^{14}\text{CO}_2$ into ^{14}C soluble and insoluble metabolites in *Vigna sinensis* and *Zea mays*. The data presented are the means of triplicate sample determinations. LSD, least significant difference values at 5% level.

Days after treatment	0	3	7	10	14	17	21
^{14}C soluble metabolites ($\text{cpm} \times 10^{-3} \text{ g}^{-1} \text{ fresh tissue min}^{-1}$)							
<i>Vigna sinensis</i>							
Control	1492	1563	1664	1701	1788	1842	1879
Waterlogging	1492	1528	1532	1596	1622	1675	1699
Salinity	1492	1512	1569	1653	1684	1756	1766
Waterlogging + Kinetin	1492	1577	1638	1735	1777	1822	1856
Salinity + Kinetin	1492	1523	1602	1658	1729	1791	1823
LSD at 5%		98.2 94.3	92.8		141.4 152.3	171.1	
<i>Zea mays</i>							
Control	1603	1869	1978	2099	2184	2322	2405
Water logging	1603	1793	1853	1949	2015	2091	2133
Salinity	1603	1739	1799	1841	1844	1953	2037
Water logging + Kinetin	1603	1822	1928	1981	2049	2259	2364
Salinity + Kinetin	1603	1802	1899	1936	2102	2238	2339
LSD at 5%		121.1	161.9	199.4	160.3	233.8	251.6
^{14}C insoluble metabolites ($\text{cpm} \times 10^{-3} \text{ g}^{-1} \text{ fresh tissue min}^{-1}$)							
<i>Vigna sinensis</i>							
Control	240	243	245	250	252	253	254
Waterlogging	240	241	241	241	243	246	245
Salinity	240	239	242	244	246	248	250
Waterlogging + Kinetin	240	245	247	249	252	252	252
Salinity + Kinetin	240	242	243	246	247	249	251
LSD at 5%		4.2	7.3	6.4	8.3	7.4	9.3
<i>Zea mays</i>							
Control	258	272	287	288	296	302	305
Water logging	258	268	274	285	296	304	307
Salinity	258	262	270	279	284	294	301
Water logging + Kinetin	258	269	278	287	296	301	302
Salinity + Kinetin	258	264	285	290	292	295	296
LSD at 5%		9.4	12.2	13.7	14.5	11.6	12.2
Sum of soluble and insoluble metabolites							
<i>Vigna sinensis</i>							
Control	1732	1806	1909	1951	2040	2095	2133
Waterlogging	1732	1769	1773	1837	1865	1921	1944
Salinity	1732	1751	1811	1897	1930	2004	2016
Waterlogging + Kinetin	1732	1822	1885	1984	2029	2074	2018
Salinity + Kinetin	1732	1765	1845	1904	1976	2040	2074
<i>Zea mays</i>							
Control	1861	2141	2265	2387	2480	2624	2710
Water logging	1861	2061	2127	2234	2311	2395	2440
Salinity	1861	2001	2069	2120	2128	2247	2338
Water logging + Kinetin	1861	2091	2206	2268	2345	2560	2666
Salinity + Kinetin	1861	2066	2184	2226	2394	2533	2635

comparable to those of controls. On the contrary, kinetin appeared to compensate for the great reduction in sucrose content caused by salinity. In roots of both plants, waterlogging had a cumulative effect on sucrose content during the whole experiment but salinity had a general reducing effect. Application of kinetin seemed to counteract the differential effects of both treatments; lowered the increases by waterlogging and raised the decreases by salinity. Polysaccharides content of shoots was either significantly decreased in response to waterlogging or unaffected by salinity in *Vigna sinensis* but significantly decreased by both treatments in *Zea mays* (Fig. 5). Similarly waterlogging in general significantly reduced polysaccharides content in roots

while salinity induced significant decreases only in *Vigna sinensis*. Application of kinetin greatly counterbalanced the reduction effect of both treatments upon polysaccharides content in shoots and roots of both plants.

The results reveal increases in contents of glucose and sucrose in logged plants mostly accompanied by decreases in polysaccharides. Much carbohydrate accumulation in roots but not in shoots of *Zea mays* plants can be attributed to disturbed translocation and respiration rates under flooding conditions. El-Shahaby *et al.* (1994) detected a decline in total carbohydrate pools of *Vicia faba* in response to excessive water supply. Gao *et al.* (1998) reported that salinity enhanced hexose accumulation in tomato. In addition,

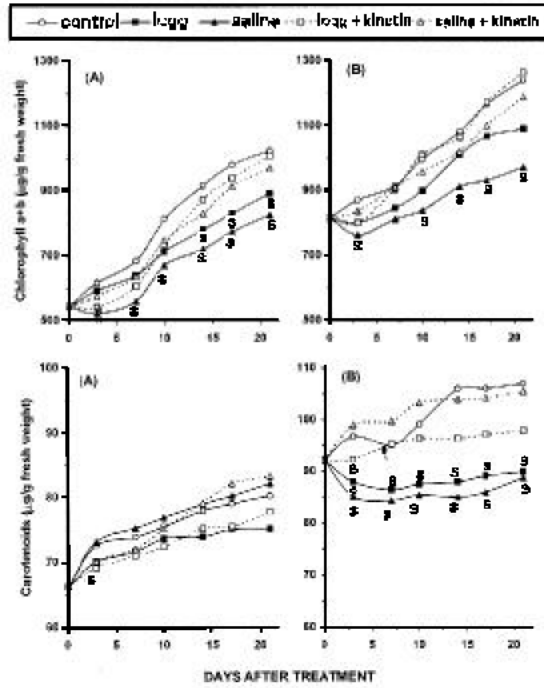


Fig. 1: The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on contents of chlorophyll a+b and carotenoids of *Vigna sinensis* (A) and *Zea mays* (B). The data presented are the means of triplicate sample determinations. S, significantly different from control at 5% level.

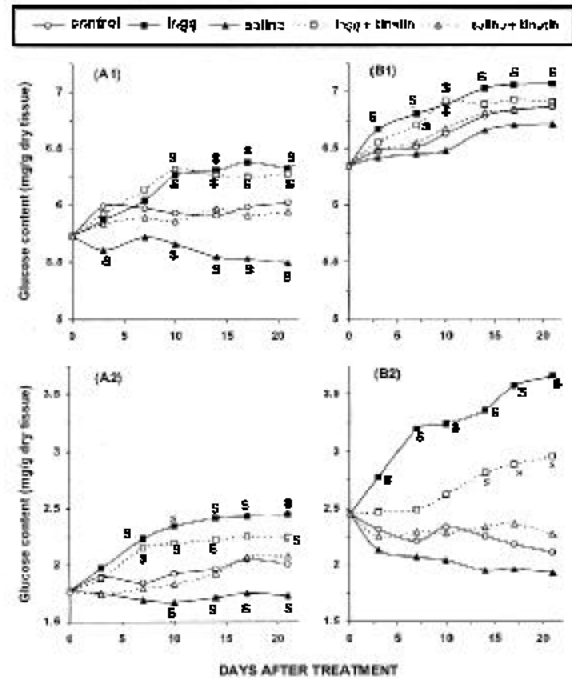


Fig. 3: The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on glucose contents of shoots (1) and roots (2) of *Vigna sinensis* (A) and *Zea mays* (B). The data presented are the means of triplicate sample determinations. S, significantly different from control as 5% level.

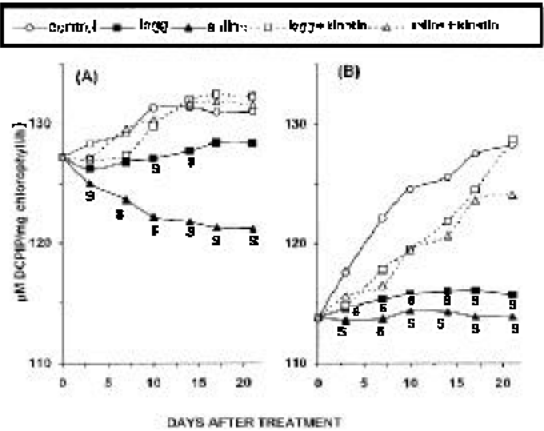


Fig. 2: The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on 2,8-dichlorophenolindodophenol (2,8-DCPIP) photoreduction by shoots of *Vigna sinensis* (A) and *Zea mays* (B). The data presented are the means of triplicate sample determinations. S, significantly different from control at 5% level.

Younis *et al.* (1993) stated that increasing NaCl concentration caused a progressive decrease in polysaccharides and total saccharides content in both shoots and roots of *Phaseolus*

vulgaris seedlings. Similarly, Mehta *et al.* (1993) found that salinity initially induced an increase in carbohydrate content followed by a decrease. Aly (1995) also found that carbohydrate contents of leaf tissues of *Magnifera indica* were decreased with increasing salinity of irrigation water. The reduction in polysaccharides induced by the stress treatments was greatly counterbalanced by kinetin. In support of these findings, spraying kinetin, IAA or GA₃ to the plants stressed by salinity resulted in increased carbohydrate contents (El-Lawendy, 1985). In the same pattern, Abdalla *et al.* (1992) stated that foliar application of 10 ppm benzyl adenine, 1 ppm 2,4-D and 50 ppm GA₃ increased markedly the values of reducing sugars, sucrose, polysaccharides and total sugars in leaves of red radish plants grown under stress conditions. This enhancement would eventually substantiate the amounts of soluble and insoluble sugars in the carbohydrate pool resulting in alleviation of the injurious effects induced by water stress. The decreased levels of these products of photosynthesis could come therefore from a reduced efficiency of photosynthetic rate that is derived by activities of photosynthetic enzymes.

The observed depression in carbohydrate fractions of *Vigna sinensis* and *Zea mays* plants in response to salinity again, appears to coincide with the decrease in photosynthetic efficiency and enzyme activities. Rubisco in shoots of *Vigna sinensis* was insignificantly changed by waterlogging but significantly decreased by salinity while it was decreased in *Zea mays* by both treatments (Fig. 6). The degree of inhibition of the enzyme activity was greater in response to salinity

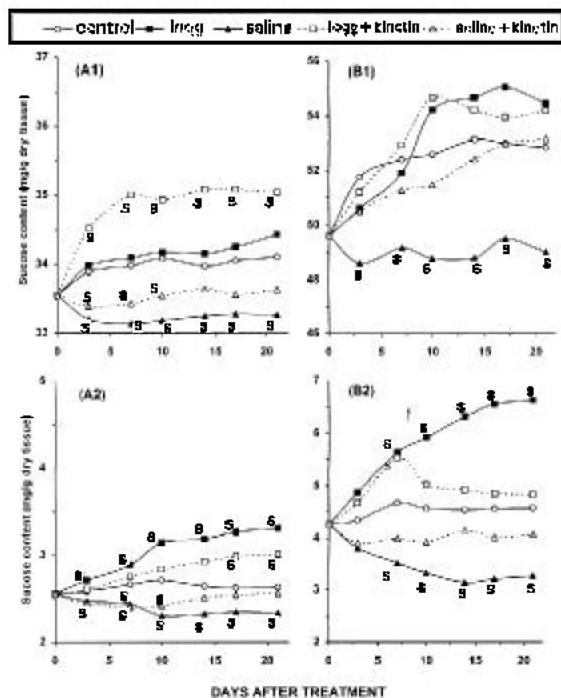


Fig. 4: The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on sucrose contents of shoots (1) and roots (2) of *Vigna sinensis* (A) and *Zea mays* (B). The data presented are the means of triplicate sample determinations. S, significantly different from control at 5% level.

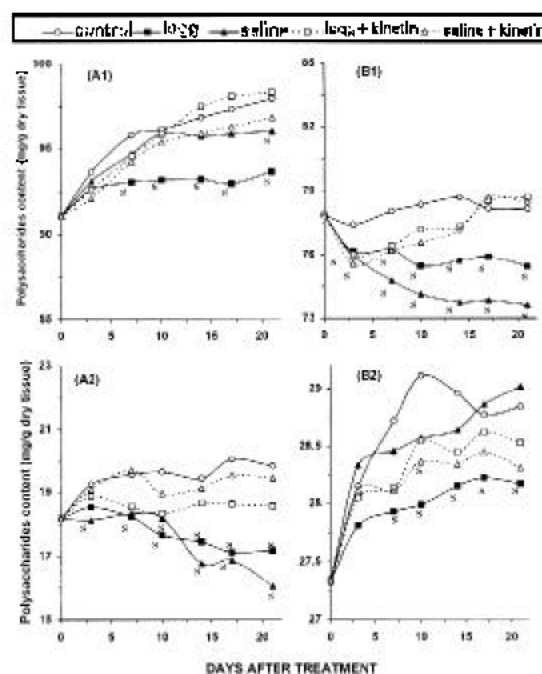


Fig. 5: The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on polysaccharides content of shoots (1) and roots (2) of *Vigna sinensis* (A) and *Zea mays* (B). The data presented are the means of triplicate sample determinations. S, significantly different from control at 5% level.

than to waterlogging. The combination of kinetin with the treatments partially nullified the inhibitory effect of these treatments on the enzyme activity of both plants. Though the enzyme activity in the treated samples was variably increased, yet levels remained lower than controls.

The results thus showed that the changes in carbohydrate fractions are presumably related to the changes in Rubisco activity. This may conclude that the inhibition in photosynthetic efficiency was accompanied by inhibited carbohydrate biosynthesis. In this context the overall growth reduction, is probably due to higher sensitivity of photosystem II, decrease of CO₂ in the intercellular spaces of stomata, reduction of photochemical quantum efficiency of CO₂ uptake, low level of oxygen evolution and low level of 3-phosphoglycerate (Seemann and Critchley, 1985; Bruguoli and Lauteri, 1991). This reflects the reduction in carbon allocation to new leaves and longer term potential photosynthetic capacity; resulting in reduction of photon yield of CO₂ assimilation and consequently limit starch synthesis in saline-stressed plants. Nevertheless, there was a reduction in the enzyme activity might be caused from a drop in its synthesis, structural integrity and/or changes in substrate generation. In this respect, Bradford (1982) reported that Rubisco was generally lowered in flooded plants.

Moreover, an increase in substrate concentration in the reaction medium of a substrate-stabilized enzyme, however, gave not only a reduction in its salt sensitivity, but also stimulated its activity under high salt conditions (Shomer-Ilan

and Waisel, 1986). They found that at low PEP concentration in the reaction media, NaCl inhibited the activity of PEP-C extracted from two C₄ halophytes. In confirmation Seemann and Critchley (1985), working on beans, noted a reduction in Rubisco *in vivo* under salinity stress. Likewise, Stiborova *et al.* (1987) using barley and maize found that salinity induced decreases in chlorophyll content and in Rubisco activity in barley as well as decreases in PEP-C and NADP-MDH in maize. It is apparent that there is a close relationship between the changes in photosynthetic activity and Rubisco activity. Thus, the predominant reduction in photosynthetic activity of both plants by waterlogging or salinization can be attributed to the reduction in the activity of Rubisco. In this connection Castrillo and Trujillo (1994) demonstrated that Rubisco activity in two cultivars of French bean plants decreased when plants were subjected to water stress. In addition, Dua *et al.* (1994) recognized that under a moderate stress, the net photosynthetic rate decreased to 50% and the activities of ribulose-5-P kinase and 3-phosphoglycerate kinase were reduced to 55% and 79% respectively. They further found that under a severe water stress, all the above characteristics were strongly reduced in addition to the activities of photosystems I and II and of the photosynthetic reduction cycle. Also, salinity induced reductions in the photosynthetic rate of *Argyranthemum coronopifolium* (De Herralde *et al.*, 1998) and *Avicenna germinans* (Pezeshki *et al.*, 1997).

In conclusion, perusal of the results may lead to a suggestion that waterlogging or salinity affected photosynthetic activity

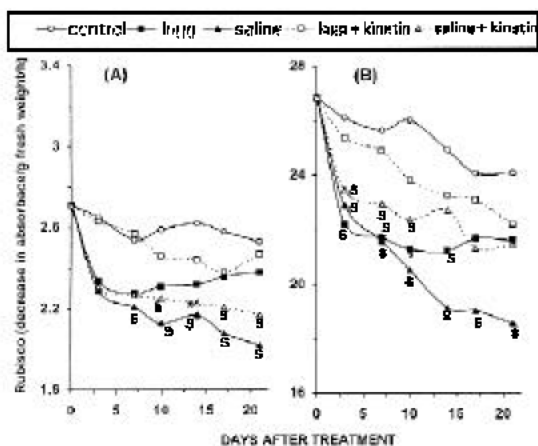


Fig. 6: The influence of waterlogging or salinity without or with the exogenous application of 50 ppm kinetin on the activity of Rubisco in shoots of *Vigna sinensis* (A) and *Zea mays* (B). The data presented are the means of triplicate sample determinations. S, significantly different from control at 5% level.

of *Vigna sinensis* and *Zea mays* might be in part due to the malfunction of photosynthetic pigments and Rubisco enzyme as well as Hill reaction. These disturbances would give rise to a reduced rate of carbohydrate formation. However, the increased levels of glucose and sucrose in the stressed plants along with a drop in polysaccharides pool might indicate that these increased levels could arise from a block in their transport rather than from an over production. Moreover, it could be suggested that exogenous application of kinetin to the stressed *Vigna sinensis* and *Zea mays* plants increased the resistance of these plants to stress conditions probably by a delay in senescence and/or an enhanced synthesis of Rubisco.

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